Toward More Efficient Clinical Trials in Motor Neuron Disease – Total Drug Exposure Analysis and Bulbar Biomarker Development

Motor neuron disease affects upper motor neurons in the brain and lower motor neurons in the spine and causes muscle weakness, atrophy, difficulty swallowing, respiratory failure, and death. The development of effective interventions for adult motor neuron diseases, such as amyotrophic lateral sclerosis (ALS) and spinal and bulbar muscular atrophy (SBMA, also known as Kennedy's disease), remains a challenge. To date, many clinical trials have been conducted and most of them have failed. In this thesis, we analyzed a previous large ALS clinical trial using total drug exposure and searched for biomarkers that correctly reflect dysphagia in SBMA to help achieve more efficient clinical trials for motor neuron disease in the future.

In my first article, we used a database of the clinical trial of ceftriaxone. Ceftriaxone, a β-lactam antibiotic, has been shown to increase the glutamate transporter that clears glutamate from the synapse. In the clinical trial of ceftriaxone for ALS, 514 participants in the United States and Canada were enrolled. Of these, 341 participants were randomly allocated to ceftriaxone and 173 to placebo for at least 12 months. The trial did not show significant clinical efficacy; however, treatment adherence may have masked potential efficacy. We analyzed the effect of total drug exposure using a random slopes regression model that included age, sex, disease duration, riluzole, El Escorial Criteria, and baseline functional scores as fixed effects and intercept and slope as random effects. We found total ceftriaxone exposure analysis did not show overall efficacy of ceftriaxone. We concluded that failed clinical trials with intervention-adherence problems may need total drug exposure analysis to assess potential effect of the medication.

In my second article, we analyzed the results of the videofluoroscopic swallowing study (VFSS) in 111 consecutive genetically confirmed patients with SBMA and 53 age- and sex-

matched healthy controls. Difficulty swallowing (dysphagia) is a major symptom of motor neuron disease, and often leads to life-threatening events such as aspiration pneumonia and suffocation. Although bulbar dysfunction is an important prognostic factor, reliable outcome measures have not been fully established to evaluate dysphagia in motor neuron disease. Out of more than 40 radiographic symptoms, the most pertinent abnormal findings in patients with SBMA include *vallecular residue after swallow* (residue just behind the tongue base)*, nasal penetration,* and *insufficient tongue movement* ($P < 0.001$ for each) as compared with healthy controls. Quantitative analyses showed *pharyngeal residue after initial swallowing, oral residue after initial swallowing, multiple swallowing sessions,* and *penetration–aspiration scale* were significantly worse in patients ($P \leq 0.005$ for each) as compared with healthy subjects. In patients with SBMA, laryngeal penetration was observed more frequently in subjects without subjective dysphagia. We revealed that dysphagia of SBMA is characterized by impaired tongue movement and nasal penetration, followed by pharyngeal residues, resulting in multiple swallowing sessions and laryngeal penetration. We concluded that *pharyngeal residue* and *penetration-aspiration scale* reflect major features of dysphagia in patients with SBMA.

Clinical trial of ceftriaxone for ALS:

Post-hoc analysis using total drug exposure

Haruhiko Banno, MD, PhD[1,2], Akihiro Hirakawa, PhD[3], Hong Yu[1],

David Schoenfeld, PhD[4], Merit E Cudkowicz, MD, MSc[1], James D Berry, MD, MPH[1]

1 Neurological Clinical Research Institute, Massachusetts General Hospital, 165 Cambridge Street, Boston, MA 02114, USA

2. Department of Neurology, Nagoya University Graduate School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya 466-8550, Japan

3. Biostatistics and Bioinformatics Section, Center for Advanced Medicine and Clinical Research, Nagoya University Hospital, 65 Tsurumai-cho, Showa-ku, Nagoya, 466-8550, Japan

4. Biostatistics Center, Massachusetts General Hospital, 50 Staniford St. Suite 560, Boston, MA 02114, USA

Correspondence to: Dr. Berry, Neurological Clinical Research Institute, Massachusetts General Hospital, 165 Cambridge Street, Boston, MA 02114, USA

E-mail: jdberry@mgh.harvard.edu

Running title: Ceftriaxone total drug exposure

Key words: amyotrophic lateral sclerosis, total drug exposure, clinical trial

Abbreviations: ALS = Amyotrophic lateral sclerosis; ALSFRS-R = Amyotrophic Lateral Sclerosis Functional Rating Scale-revised. 31 references, 4 figures, 1 table, 1 supplementary figure, 2 supplementary tables, 1 supplementary method.

Word count: Title, 71 characters; Abstract, 250 words; Text, 2865 words

ABSTRACT

Objective Ceftriaxone, a β-lactam antibiotic, has been shown to increase the mRNA for a glutamate transporter that clears glutamate from the synapse. In a clinical trial of ceftriaxone, 514 patients with ALS were enrolled in an efficacy study. The trial did not show significant clinical efficacy; however, treatment adherence may have masked potential efficacy. We sought to assess whether ceftriaxone total drug exposure had any effect on the rate of decline in function compared with placebo through 1-year follow-up.

Methods The outcome measures for post-hoc analysis were the same as those in the primary trial: difference between the treatment and placebo groups in rate of decline in function as measured by ALSFRS-R; secondary outcome measures included vital capacity and muscle strength. We set total drug exposure as a time-varying covariate. The effect of total drug exposure was analysed using a random slopes regression model that included age, sex, disease duration, riluzole, El Escorial criteria and baseline functional scores as fixed effects and intercept and slope as random effects.

Results Of the participants in the ceftriaxone arm, 42.2 percent received less than half the full dosage for 1 year. No favorable correlation was found between total ceftriaxone exposure and ALSFRS-R changes. Total drug exposure analysis using the random slopes regression model did not show any effect ($p = 0.714$).

Conclusions Total drug exposure analysis did not show overall efficacy of ceftriaxone. Failed clinical trials with intervention-adherence problems may benefit from total drug exposure analysis to assess potential effect of the medication.

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a progressive and fatal neuromuscular disease that affects mainly motor neurons of the brain and spine and results in progressive muscle weakness, atrophy, and respiratory failure. [1,2] ALS is more common in people 60 to 69 years of age than in other age groups, and the prevalence of patients with a definite diagnosis of ALS is 3.9 cases per 100,000 people in the US general population.[3] Median survival was reported to be 36 months after the first symptom, although 10 percent of the patients were alive more than 10 years later.[4] Current approved medications, riluzole, and edaravone in Japan, have had limited impact on disease progression.[5,6] With the recent progress in causative gene discoveries in ALS[7,8] and clinical trials of gene silencing and stem cell transplantation,[9,10] increasing attention has focused on ALS therapy development. Many clinical trials have been performed and most of them have failed to show effective results. The reasons for the futility of these trials are multifaceted: the central disease mechanism that causes neurodegeneration is unclear; animal models that correctly recapitulate human disease mechanisms, especially of sporadic ALS cases, are lacking; disease heterogeneity, both genetically and phenotypically, is a challenge; a high participant drop-out rate in clinical trials due to short life expectancy is common, and target biomarkers are lacking.[11] Failed clinical trials may, however, suggest important avenues to future success.

Ceftriaxone, a β-lactam antibiotic, has been shown to increase the level of a glutamate transporter that clears glutamate from the synapse and decreases glutamate excitotoxicity in rodent brains and in human astrocyte cultures.[12-14] Ceftriaxone also slowed disease progression and prolonged survival in a mouse model of ALS.[13]

In the clinical trial of ceftriaxone for ALS, 514 patients with ALS in the United States and Canada were enrolled.[15] Of these, 341 participants were randomly allocated to ceftriaxone and

173 to placebo for at least 12 months. All participants were asked to stay in the study until the last enrolled participant completed 12 months of treatment. On average, participants receiving ceftriaxone remained on drug for a mean of 14.2 months (SD 11.7), whereas participants receiving placebo remained on drug for 12.3 months (SD 9.2). Drug exposure variance was also caused by the trial suspension due to futility, because of failure of the drug to show significant efficacy. However treatment adherence may have masked the potential efficacy of ceftriaxone because long treatment duration and adverse events can result in suspending or discontinuing the medication. In this post-hoc study, which focused on total drug exposure, our objectives were as follows: (1) to assess whether ceftriaxone total drug exposure had any positive effect on the rate of slope decline in function compared with the placebo arm; (2) to describe the potential effect of total ceftriaxone exposure; (3) to explore the potential effect of ceftriaxone by missing data analysis.

METHODS

Ceftriaxone trial

We conducted a post-hoc analysis of a randomised, double-blinded, placebo-controlled, multicenter, phase 3 trial of the safety and efficacy of ceftriaxone versus placebo for the treatment of ALS, which was conducted at 59 clinical sites in the United States and Canada between 2006 and 2012.[15] This study originally consisted of three stages: stage 1 (pharmacokinetics in the plasma and cerebrospinal fluid); stage 2 (safety over 20 weeks); and stage 3 (efficacy).[16] In stage 3, new participants were randomly assigned (2:1) to 4 grams of ceftriaxone or placebo. Participants who were in stage 1 and 2 were also randomly allocated (2:1) to ceftriaxone or placebo at each stage and continued the trial medication in a blinded

fashion at stage 3.[15] For the ceftriaxone group participants in stage 3, which we analysed in this post-hoc analysis, 2 grams of ceftriaxone or placebo were administered twice daily to the patient through a central venous catheter by a trained caregiver in the home. The last participant enrolled in December 2011, but the study was stopped for futility by the data safety monitoring board in July 2012 before all patients had received full treatment.[15] This clinical trial protocol was approved by the institutional review board at each participating centre, and all participants provided written informed consent. The trial is registered at ClinicalTrials.gov, number NCT00349622. The study was performed in accordance with the Declaration of Helsinki and Good Clinical Practice. This post-hoc analysis was approved by the steering committee of the clinical trial.

Outcome measures

The primary outcome measures for this post-hoc analysis were the same as those in the primary trial: difference between the treatment and placebo groups in rate of decline in function as measured by ALS Functional Rating Scale-revised (ALSFRS-R).[17] Secondary outcome measures included vital capacity, muscle strength measured using handheld dynamometry (HHD), the Amyotrophic Lateral Sclerosis Specific Quality of Life (ALSSQOL) questionnaire[18] and the Caregiver Burden Inventory (CBI) [19] (Supplementary methods).

Total drug exposure

Total drug exposure in ceftriaxone group participants was calculated by the sum of daily exposure to ceftriaxone or placebo, which ranged from 0 gram to 4 grams a day. We calculated the total amount of ceftriaxone during the 12-month clinical trial observation period, even though

some patients had drug holidays because of side effects, etc. The maximum dosage of ceftriaxone for 1 year was 1440 grams (4 grams*30 days*12 months). In the placebo group, participants were given a paediatric multivitamin for infusion to colour-match the ceftriaxone syringes. The amount of placebo infusion was used for comparison in total drug exposure analyses.

Statistical methods

We used the same data set as the primary analyses of the ceftriaxone trial. First, we reconfirmed the primary analysis results, which were based on the intention-to-treat principle. Second, a histogram, a scatter plot and a three-dimensional plot regarding total drug exposure were constructed (figure 1, 2 and 3). Third, the effect of total drug exposure was analysed using a random slopes regression model that included age, sex, disease duration, riluzole, El Escorial Criteria and a baseline functional score as fixed effects and intercept and slope as random effects (supplementary method, and table 1). Total drug exposure was set as a time-varying covariate. The slopes of the functional score (eg, ALSFRS-R) in 120 gram-unit drug exposure was compared between the two groups. The analyses were conducted only for continuous or ordinal functional variables to make them appropriate for our model. Covariance structure for random effects was set as unstructured. We analysed the relationship between mean daily functional changes and mean daily drug exposure (supplementary table 1). The effect of mean daily drug exposure was analysed using a random slopes regression model that included age, sex, disease duration, riluzole, El Escorial Criteria[20] and a baseline functional score as fixed effects and intercept and slope as random effects. The dependent variable of the mean daily drug exposure analysis is the change per day [eg, (ALSFRS-R score of any visit – ALSFRS-R score of the

previous visit) / (duration between the two visits)]. We set mean daily drug exposure (total drug exposure between the two visits) as a time-varying covariate as we did in total drug exposure analyses. Mixed-effects model repeated measures (MMRM) analysis was used to evaluate the effect of missing data and to speculate on the potential effect of ceftriaxone by comparing adjusted mean of ceftriaxone arm with that of placebo arm at each time point (figure 4, supplementary table 2 and supplementary figure). All the statistical analyses were performed using SAS (version 9.4; SAS Institute Cary Inc., CA, USA). For all tests, a p value of <0.05 was considered statistically significant.

RESULTS

Distribution of total ceftriaxone exposure

Of 340 analysed participants in the ceftriaxone arm, 184 discontinued the study (84 because of an adverse event, 31 because of disease progression); 156 completed the study. Of 173 analysed participants in the placebo arm, 102 discontinued the study (32 because of an adverse event, 16 because of disease progression); 71 completed the study. Of those receiving ceftriaxone, 22.7 percent of the patients had 0-360 grams of drug exposure (0 to 3 months equivalent dosage), 19.5 percent of the patients had 361-720 grams of drug exposure (3 to 6 months equivalent dosage), 21.8 percent of the patients had 721-1080 grams of drug exposure (6 to 9 months equivalent dosage), and 38.4 percent of the patients had 1081-1440 grams of drug exposure (9 to 12 months equivalent dosage) for the 1-year follow-up duration. Dose distribution of ceftriaxone group was similar to that of placebo group (figure 1).

ALSFRS-R changes and total ceftriaxone exposure

If the total ceftriaxone exposure affects functional severity positively in spite of disease course, the ALSFRS-R changes at drug suspension will be less severe in the patients with high total exposure. We calculated the ALSFRS-R changes in participants in the ceftriaxone arm (n = 340) and presented the results in a scatter plot with total ceftriaxone exposure. No positive correlation was found between total ceftriaxone exposure and ALSFRS-R changes at drug suspension (r^2 = 0.081; figure 2).

Three dimensional plot of total drug exposure, follow-up time and ALSFRS-R changes

We analysed the difference between the participants in the ceftriaxone arm and those in the placebo arm using total drug exposure, time of drug suspension and ALSFRS-R changes at the drug suspension. We used time of drug suspension instead of 12 months (full observation duration) to find out the effect of total drug exposure on both the ALSFRS-R changes and drug suspension. The three-dimensional plot shows the relationships among three parameters: time of drug suspension (X-axis), total ceftriaxone exposure (Y-axis), and ALSFRS-R changes at the drug suspension (Z-axis) (figure 3A). The overall contours of the ceftriaxone group and the placebo group were almost identical, even though participant numbers differ between two groups, indicating that total ceftriaxone exposure is not affecting ALSFRS-R changes at the drug suspension compared with the placebo arm (figure 3 B, C).

Total drug exposure analysis using a random slopes regression model

Total ceftriaxone exposure analysis using a random slopes regression model of decline in function as measured by ALSFRS-R that includes age, sex, disease duration, riluzole, El Escorial

Criteria and a baseline functional score did not show potential efficacy (p = 0.714, table 1A). Table 1B shows similar analyses for secondary endpoints including vital capacity, HHD muscle strength of upper extremities and lower extremities, ALSSQOL and CBI. Only CBI showed a worsening trend in the ceftriaxone arm (p = 0.062).

Mean daily drug exposure analysis using a random slopes regression model

We analysed the relation between "mean daily ALSFRS-R change between any two visits: (ALSFRS-R change) / (days between two visits)" and "mean daily drug exposure: (drug exposure between the visits) / (days between two visits)". We did this analysis because total ceftriaxone exposure might be affected by drug suspensions at drug holidays or because of temporary side effects. Mean daily drug exposure analysis using a random slopes regression model of decline in function as measured by ALSFRS-R that includes age, sex, disease duration, riluzole, El Escorial Criteria and baseline ALSFRS-R score did not show significant efficacy (p = 0.066, supplementary table 1). No significant efficacy was shown in similar analyses for secondary endpoints (supplementary table 1).

Mixed-effects model repeated measures (MMRM) analysis

We evaluated the effect of missing data and speculated on the potential effect of ceftriaxone. By analyzing ALSFRS-R changes per month for 6 and 12 months, using MMRM, a significant difference of least square mean was found only at the point of 12 months (p = 0.038, figure 4). Otherwise, no significant differences were found in other endpoints (supplementary supplementary table 2).

DISCUSSION

In this study, our goal was to assess whether ceftriaxone total drug exposure had any effect on function by post-hoc analyses. First, we found that total drug exposure of ceftriaxone group was similar to that of placebo group. Of the participants in the ceftriaxone arm, 42.2 percent received less than half the full dosage for 1 year. Second, we could not find any positive correlations between total ceftriaxone exposure and ALSFRS-R changes at drug suspension. Third, the three-dimensional plot of time of drug suspension (X-axis), total ceftriaxone exposure (Y-axis), and ALSFRS-R changes at the drug suspension (Z-axis) showed no obvious morphological differences between participants in the ceftriaxone arm and those in the placebo arm. Fourth, total drug exposure analysis using a random slopes regression model that included age, sex, disease duration, riluzole, El Escorial Criteria and baseline ALSFRS-R score did not show significant potential efficacy of ceftriaxone. We found that the Caregiver Burden Inventory (CBI) was worse in the ceftriaxone arm by total drug exposure analysis. This result may be explained by diarrhea, a common side effect of ceftriaxone that results in further burdens for caregivers. Last, we also explored the potential effect of ceftriaxone by missing data analysis. Mixed-effects model repeated measures (MMRM) analysis revealed significant slope difference only at the point of 12 months ($p = 0.038$). Given the results of the other analyses, excepting MMRM, we concluded the overall ceftriaxone total drug exposure had no significant effect in patients with ALS.

Recently, MMRM has become commonly used for missing data analyses. In a sensitivity analysis of 48 clinical trial datasets obtained from 25 New Drug Applications submitted for neurological and psychiatric drug products, MMRM analysis appears to be a superior approach in controlling false-positive error rates and minimizing biases, as compared with last observation

carried forward (LOCF) analysis of covariance.[21] Our data showed marginal significance only at 12 months and no significant efficacy was detected at other time points (supplementary table 2). Given that this is a post-hoc analysis, our evaluation of the overall statistical results of this medication is that it is not potent enough to induce significant clinical efficacy.

The reasons for failure in clinical trials for patients with ALS can be classified by several factors. First, superoxide dismutase-1 (SOD1) transgenic rodent models do not recapitulate sporadic disease in humans and minimally replicate mutant *SOD1* familial ALS.[22, 23] Further, the central pathophysiology of neurodegeneration in ALS remains unknown.[24] In addition, we should consider pharmacological aspects of the investigational medication, such as interaction of riluzole, appropriate dosage, pharmacokinetic or pharmacodynamics markers, cerebrospinal fluid penetration or bioavailability, and biomarker relevance.[11,25] In terms of trial design, disease heterogeneity and differences in study population between phase 2 and phase 3 trials can cause trial failures.[26] Data from failed clinical trials is valuable to revisit because of the potential for participant enrichment, which may contribute to the success of future studies. This was seen in the case of edaravone, which was recently approved in Japan after employing a strict enrichment strategy.[27] In addition, potential biomarkers can be found in data from previous trials.[26]

The ceftriaxone clinical trial in ALS was conducted at more than 50 centres and with more than 500 participants. Intravenous infusion of ceftriaxone was performed twice daily through a central venous catheter at home for at least 12 months. Given the daily multiple infusions for the long duration and the risk of catheter infection, cholelithiasis[28] and renal dysfunction[29] caused by ceftriaxone, total drug exposure varies for the individual patient, which we confirmed in our study. Drug exposure variance was also a result of the trial suspension due to futility. Therefore, the potential effect of ceftriaxone should be reevaluated by total drug

exposure; our current study revealed no significant effects by relevant analyses, except for the missing data analysis using MMRM. These results suggest that ceftriaxone tolerability and early trial suspension are not the causes of the trial failure.

The strength of this post-hoc analysis is the use of a new parameter, total drug exposure, which is closely related to drug adherence and patient retention. In Parkinson's disease, a cumulative dose of dopamine agonists correlated to vulvopathy.[30] However, use of total drug exposure in ALS clinical trials has rarely been conducted.[31] Since ALS is a life-limiting neuromuscular disease that results in progressive muscle weakness and poor quality of life, many patients in a clinical trial for ALS tend to withdraw from trial participation, decrease the dose, or stop taking the medication altogether in the midst of the study. In line with these observations, we conducted this post-hoc analysis using total ceftriaxone exposure. We successfully conducted total drug exposure analyses that did not show significant potential efficacy of ceftriaxone. The other strength of this study is that the ceftriaxone trial has long-term longitudinal data of more than 500 patients with ALS, one of the largest ALS interventional trials to date. The limitations of our study include the fact that this is a post-hoc analysis. Prespecified secondary analyses are ideal to avoid false-positive results (type I error). However, given that there are few clinically useful medications for ALS, greater effort should be devoted toward reviewing previous studies to find potential effects of the intervention and to design comparable but more efficient clinical trials for the future. We suggest that total drug exposure analysis as a potential prespecified secondary analysis would be valuable for a future clinical trial in ALS with a long follow-up duration.

In summary, our study revealed that a total ceftriaxone exposure analysis using a random slopes regression model did not show overall efficacy of ceftriaxone. Failed clinical trials with

patient adherence problems may benefit from total drug exposure post-hoc analysis to eliminate false-negative results and to elucidate the potential efficacy of the study medication.

Acknowledgments

We thank the study participants and their families and caregivers. Douglas Hayden gave us technical advice. This work was funded by the Harvard Catalyst, the Uehara Memorial Foundation and the Toyoaki Foundation.

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Figure legends

Figure 1 Distribution of total ceftriaxone exposure

Of 340 analysed participants in the ceftriaxone arm, 22.7 percent of the patients had 0 to 3 months equivalent dosage, 19.5 percent had 3 to 6 months equivalent dosage, 21.8 percent had 6 to 9 months equivalent dosage, and 38.4 percent had 9 to 12 months equivalent dosage for the 1-year follow-up duration. Dose distribution of ceftriaxone group was similar to that of placebo group.

Figure 2 ALSFRS-R changes and total ceftriaxone exposure

ALSFRS-R changes at drug suspension (n = 340) and total ceftriaxone exposure shown in a scatter plot. No positive correlation was found between total ceftriaxone exposure and ALSFRS-R changes at drug suspension ($r^2 = 0.081$).

Figure 3 Three-dimensional plots of total drug exposure, follow-up time and ALSFRS-R changes

The three-dimensional plot shows the relationships among three parameters: time of drug suspension (X-axis), total drug exposure (Y-axis), and ALSFRS-R changes at drug suspension (Z-axis) (A). The overall contours of the ceftriaxone group and the placebo group were not morphologically different even though participant numbers are different between the ceftriaxone arm (n = 340) and the placebo arm (n = 173), indicating total ceftriaxone exposure is not affecting ALSFRS-R changes at drug suspension compared with the placebo arm (B, C).

Figure 4 Mixed-effects model repeated measures (MMRM) analysis in ALSFRS-R

The effect of missing data was analysed by the mixed-effects model repeated measures (MMRM) approach. By analysing ALSFRS-R changes per month for 6 and 12 months, a significant difference of least square mean was found only at the point of 12 months (p = 0.038).

Supplementary figure Mixed-effects model repeated measures (MMRM) analysis in secondary endpoints

The effect of missing data was analysed by the mixed-effects model repeated measures analysis. No significant differences of least square mean were found in primary and secondary endpoints (changes per month for 1, 4, 8 and 12 months). Detailed p-values are shown in supplementary table 2. ALSSQOL, Amyotrophic Lateral Sclerosis Specific Quality of Life.

Table Total drug exposure analysis using a random slope regression model as measured by ALSFRS-R (A) and secondary endpoints (B)

(A)	**Ceftriaxone n=340**	**Placebo n=173**	**Difference**	**p**
Intention-to-treat analysis (primary analysis)	-1.13 (0.04)	-1.22 (0.06)	0.09 (0.08)	0.237
Total ceftriaxone exposure analysis (post-hoc analysis)	-1.08 (0.07)	-1.04 (0.08)	-0.04 (0.11)	0.714

(B)	**Ceftriaxone n=340**	**Placebo n=173**	**Difference**	**p**
Percent vital capacity	-3.04 (0.23)	-3.49 (0.27)	0.45 (0.35)	0.201
Upper limb muscle strength	-9.99 (0.70)	-8.45 (0.83)	-1.54 (1.08)	0.155
Lower limb muscle strength	-9.13 (0.68)	-8.39 (0.85)	-0.74 (1.09)	0.498
ALSSQOL	-4.76 (0.50)	-4.22 (0.59)	-0.54 (0.77)	0.485
Caregiver Burden Inventory	1.72 (0.12)	1.37 (0.14)	0.35 (0.19)	0.062

Data are mean (standard error). The mean values are change from baseline through 1-year follow-up in units per month for intention-to-treat analysis, and per 120-gram unit exposure for total drug exposure analysis.

ALSFRS-R, Amyotrophic Lateral Sclerosis Functional Rating Scale-revised; ALSSQOL, Amyotrophic Lateral Sclerosis Specific Quality of Life.

Supplementary table 1 Mean daily drug exposure analysis using a random slope regression model as measured by ALSFRS-R and secondary endpoints

	Ceftriaxone n=340	**Placebo n=173**	**Difference**	**p**
ALSFRS-R	0.000019 (0.000109)	0.000373 (0.000159)	-0.000355 (0.000193)	0.066
% Slow vital capacity	-0.00254 (0.00104)	-0.00411 (0.00183)	0.00157 (0.00210)	0.454
Upper limb muscle strength	-0.00048 (0.00118)	0.00091 (0.00207)	-0.00139 (0.00239)	0.560
Lower limb muscle strength	-0.00056 (0.00140)	0.00046 (0.00227)	-0.00102 (0.00267)	0.701
ALSSQOL	-0.00382 (0.00189)	-0.00357 (0.00293)	-0.00025 (0.00350)	0.943
Caregiver Burden Inventory	0.00028 (0.00034)	0.00018 (0.00058)	0.00011 (0.00067)	0.871

Data are mean (standard error). The mean values are change from baseline through 1 year of follow-up per 120 g unit for total drug exposure analysis. ALSFRS-R, revised Amyotrophic lateral

sclerosis functional rating scale; ALSSQOL, Amyotrophic Lateral Sclerosis Specific Quality of Life.

Supplementary table 2 Mixed-effects model repeated measures (MMRM) approach for ALSFRS-R and secondary endpoints

	Time point	Least square mean difference	95%CI	p
ALSFRS-R	1 month	-0.354	(-1.532, 0.824)	0.556
	4 months	-0.016	(-1.218, 1.185)	0.979
	8 months	0.358	(-0.876, 1.592)	0.569
	12 months	1.376	(0.076, 2.676)	0.038
% Slow vital capacity	1 month	-0.076	(-3.519, 3.367)	0.965
	4 months	0.974	(-2.602, 4.549)	0.593
	8 months	0.252	(-3.680, 4.185)	0.900
	12 months	3.093	(-1.364, 7.551)	0.174
Upper limb muscle strength	1 month	-0.491	(-8.990, 8.008)	0.910
	4 months	-2.140	(-11.245, 6.965)	0.645

	8 months	-4.304	(-14.846, 6.238)	0.423
	12 months	2.739	(-9.580, 15.058)	0.663
Lower limb muscle strength	1 month	-1.296	(-12.004, 9.411)	0.812
	4 months	-3.449	(-14.772, 7.874)	0.550
	8 months	-5.454	(-17.888, 6.980)	0.390
	12 months	-2.219	(-16.751, 12.314)	0.765
ALSSQOL	1 month	-3.430	(-13.291, 6.432)	0.495
	4 months	-1.541	(-11.866, 8.783)	0.770
	8 months	-9.702	(-20.880, 1.475)	0.089
	12 months	-1.747	(14.197, 10.703)	0.783
Caregiver Burden Inventory	1 month	0.066	(-2.043, 2.176)	0.951
	4 months	1.209	(-0.964, 3.383)	0.275

8 months	0.794	(-1.547, 3.134)	0.506
12 months	1.418	(-1.228, 4.065)	0.293

The mean values are change from baseline through 1 year of follow-up per 120 g units.

ALSFRS-R, revised Amyotrophic lateral sclerosis functional rating scale; ALSSQOL, Amyotrophic Lateral Sclerosis Specific Quality of Life; CI, confidence interval.

Supplementary Methods

Outcome measures

ALSFRS-R is an ordinal rating scale (ratings 0 to 4) used to determine subjects' assessment of their capability and independence in 12 functional activities.[1] All 12 activities are relevant in ALS. Forty-eight is the highest number possible, and reflects the score of people in the normal healthy population. The vital capacity (VC) (percent of predicted normal) was determined, using the upright slow VC method. HHD (hand-held dynamometry) is a non-invasive device that allows measurements of isometric strength in muscle groups of the arms and legs. The ALSSQOL was developed to reflect overall QOL rather than health-related QOL and is based on the McGill Quality of Life Questionnaire. The ALSSQOL has a total of 46 items (ratings 1 to 10) that can be divided into six factors.[2] The higher the total number, the better the QOL. The CBI is a 24-item multi-dimensional questionnaire measuring caregiver burden.[3] Scores for each item are evaluated from 0 (not at all disruptive) to 4 (very disruptive). A total score >36 indicates a risk of "burning out," whereas scores near or slightly above 24 indicate a need to seek some form of respite care.

Model selection

In the primary analysis, ALSFRS-R scores were analysed for all patients in the intention-to-treat population by use of a random slopes regression model:

$$Y_{ij}=B_1trt_i+B_2trt_itime_{ij}+b_{0i}+b_{1i}time_{ij}$$

Where Y is the ALSFRS-R score, i denotes a particular patient, j indicates time. trt

indicates whether the patient received ceftriaxone (trt_i=1) or placebo (trt_i=0). The model had two fixed effect components (B_1, B_2) for each patient, with random effects (b_{0i}, b_{1i}).

In our post-hoc total drug exposure analysis, ALSFRS-R scores were also analysed by use of a random slopes regression model[4]:

$$Y_{ij}=B_1trt_i+B_2trt_i total\ drug\ exposure_{ij}+B_3 baseline\ ALSFRS\text{-}R+B_4 age+B_5 disease\ duration+B_6 sex+B_7 riluzole+B_8 El\ Escorial\ criteria+b_{0i}+b_{1i}time_{ij}$$

Total drug exposure of both ceftriaxone and placebo was substituted for time, because total drug exposure is a time-dependent variable that positively correlates with time. The model had eight fixed effect components (B_1, B_2…B_8) for each patient, with random effects (b_{0i}, b_{1i}). The effect of death filter was considered negligible because the observation period is 1 year at most, and to differentiate death filter and random dropout is not practical. As the $t_{1/2}$ of ceftriaxone is less than 12 hours, the onset of ceftriaxone action was considered to be at the same time of ceftriaxone administration. Interaction terms between time and other covariates (baseline ALSFRS-R, disease duration, etc.) were not considered, so that we could directly compare results of our model with those of the primary analysis. Higher order dose effects were also not considered for the same reason.

1. Cedarbaum JM, Stambler N, Malta E, et al. The ALSFRS-R: a revised ALS functional rating scale that incorporates assessments of respiratory function. BDNF ALS Study Group (Phase III). J Neurol Sci 1999;169:13-21.
2. Simmons Z, Felgoise SH, Bremer BA, et al. The ALSSQOL: balancing physical

and nonphysical factors in assessing quality of life in ALS. Neurology 2006;67:1659-64.

3. Novak M, Guest C. Application of a multidimensional caregiver burden inventory. The Gerontologist 1989;29:798-803
4. Cnaan A, Laird NM, Slasor P. Using the general linear mixed model to analyse unbalanced repeated measures and longitudinal data. Stat Med 1997;16:2349-80.

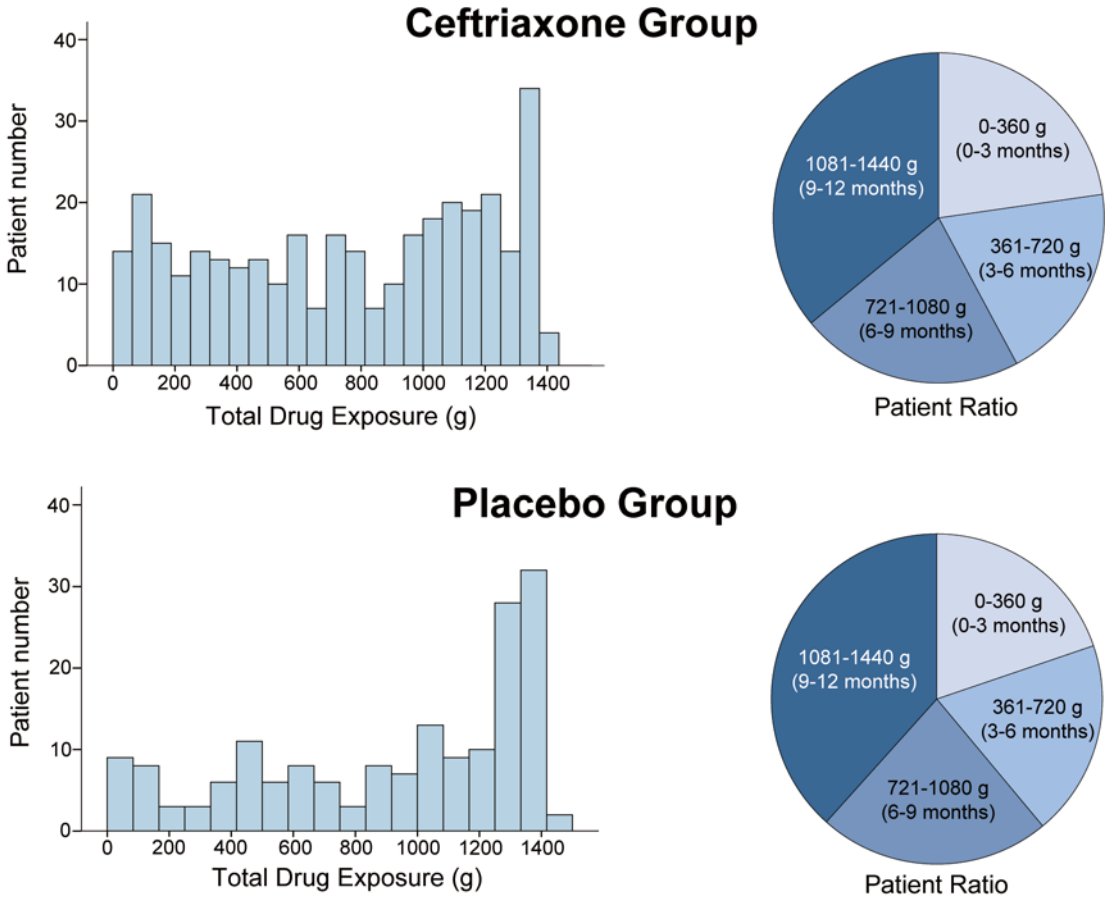

Ceftriaxone Group
Patient number
Total Drug Exposure (g)
0
200
400
600
800
1000
1200
1400
0
10
20
30
40
0-360 g
(0-3 months)
361-720 g
(3-6 months)
721-1080 g
(6-9 months)
1081-1440 g
(9-12 months)
Patient Ratio
Placebo Group
Patient number
Total Drug Exposure (g)
Patient Ratio

Figure 1

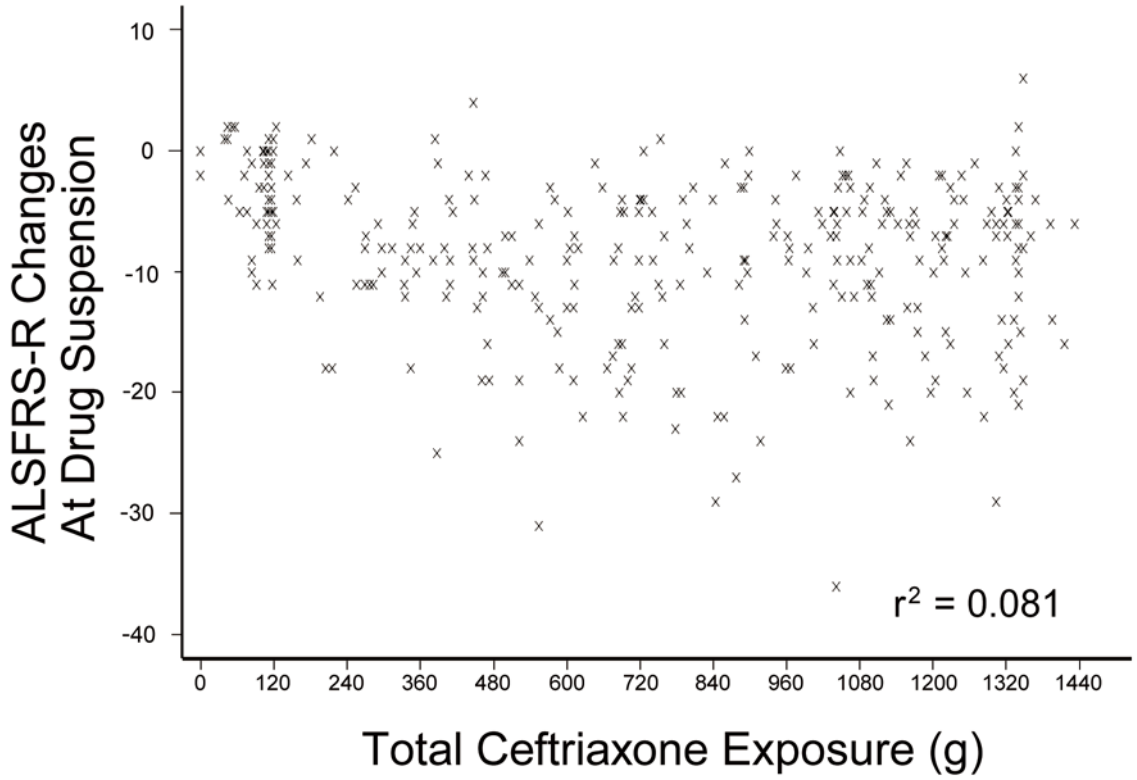

ALSFRS-R Changes
At Drug Suspension
10
0
-10
-20
-30
-40
0
120
240
360
480
600
720
840
960
1080
1200
1320
1440
r2 = 0.081
Total Ceftriaxone Exposure (g)

Figure 2

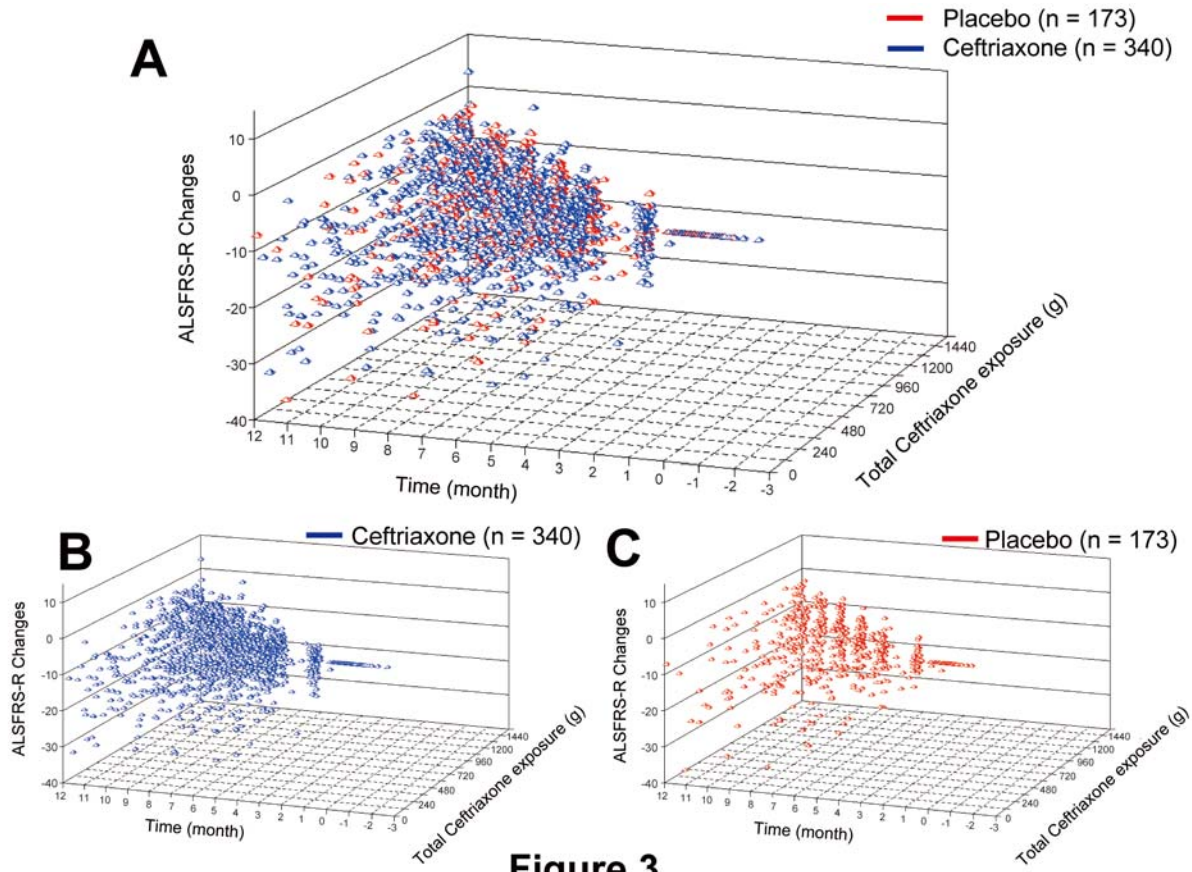

A
Placebo (n = 173)
Ceftriaxone (n = 340)
ALSFRS-R Changes
10
0
-10
-20
-30
-40
12 11 10 9 8 7 6 5 4 3 2 1 0 -1 -2 -3
Time (month)
0 240 480 720 960 1200 1440
Total Ceftriaxone exposure (g)
B
Ceftriaxone (n = 340)
ALSFRS-R Changes
Time (month)
Total Ceftriaxone exposure (g)
C
Placebo (n = 173)
ALSFRS-R Changes
Time (month)
Total Ceftriaxone exposure (g)

Figure 3

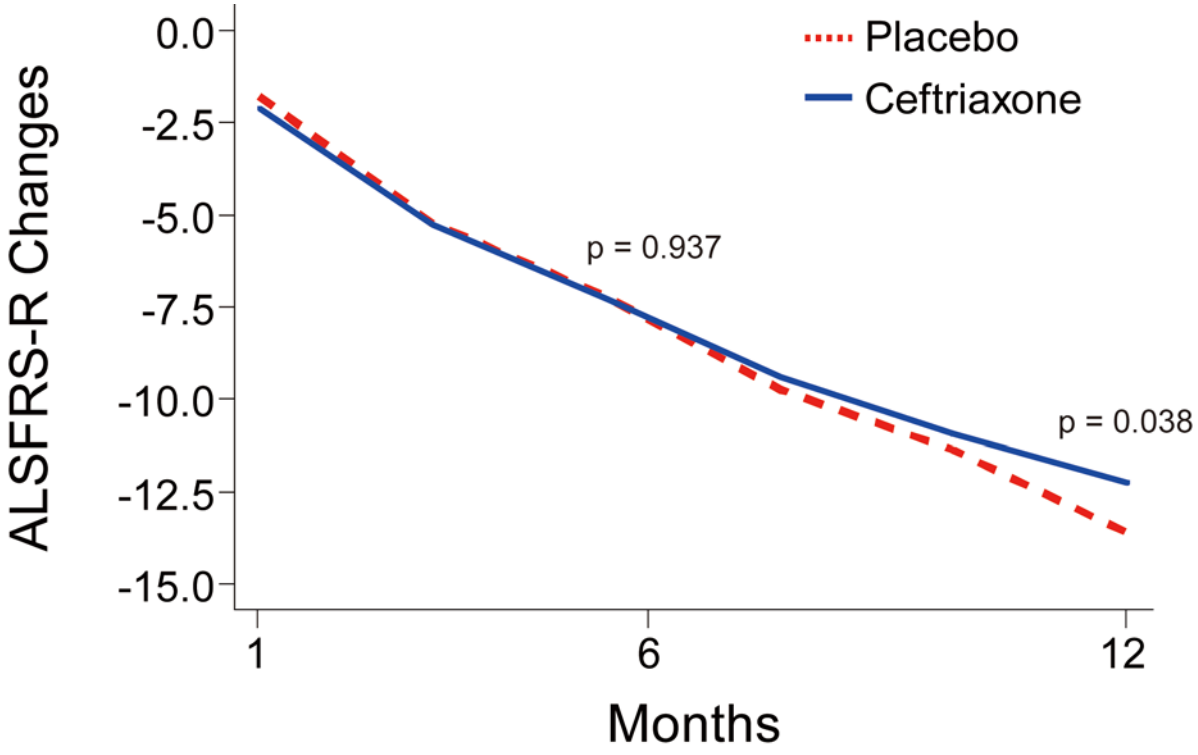
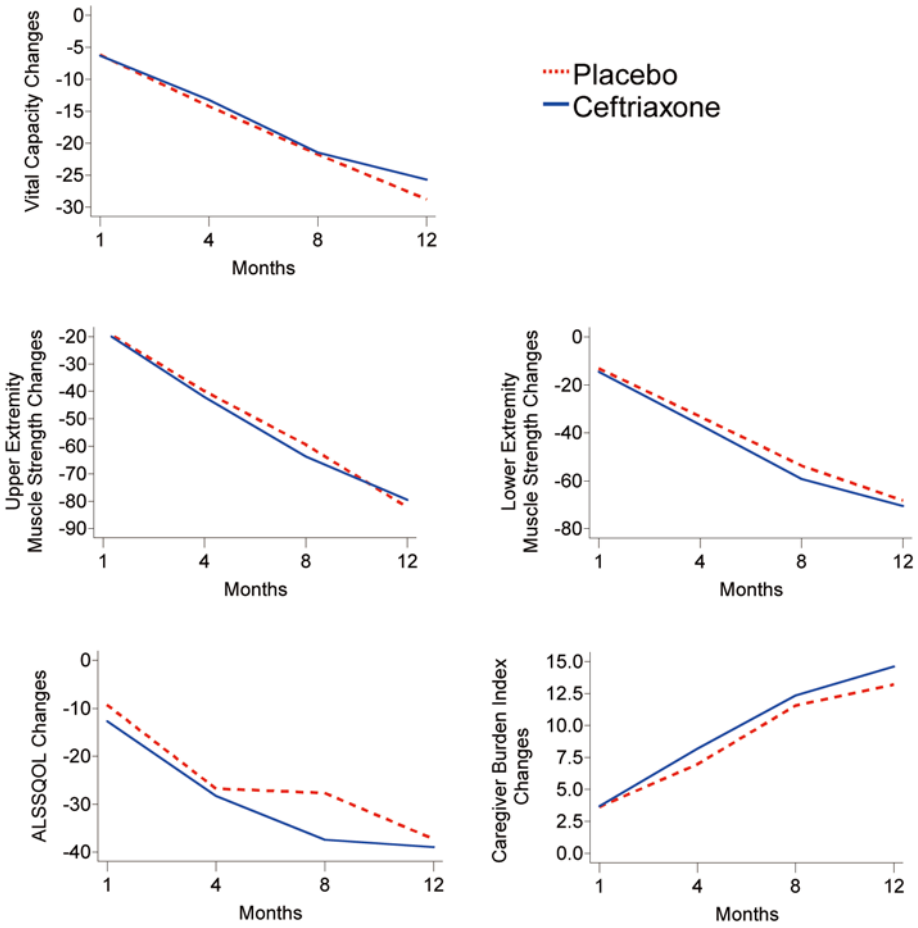

ALSFRS-R Changes
0.0
-2.5
-5.0
-7.5
-10.0
-12.5
-15.0
Placebo
Ceftriaxone
p = 0.937
p = 0.038
1
6
12
Months

Figure 4



Supplementary figure

Videofluoroscopic markers in spinal and bulbar muscular atrophy: a study of 111 patients

Haruhiko Banno, MD, PhD[1,2], Masahisa Katsuno, MD, PhD[1],
Keisuke Suzuki, MD, PhD[1,3], Seiya Tanaka, PhD, SLP[4], Noriaki Suga, MD, PhD [1],
Atsushi Hashizume, MD, PhD [1], Tomoo Mano, MD[1], Amane Araki, MD[1],
Hirohisa Watanabe, MD, PhD[1], Yasushi Fujimoto, MD, Ph.D[5],
Masahiko Yamamoto, MD, Ph.D[6], and Gen Sobue, MD, Ph.D[1,7]

1 Department of Neurology, Nagoya University Graduate School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya 466-8550, Japan

2 Neurological Clinical Research Institute, Massachusetts General Hospital, 165 Cambridge Street, Boston MA 02114, USA

3 Innovation Centre for Clinical Research, National Centre for Geriatrics and Gerontology, 7-430 Morioka, Obu 474-8511, Japan

4 Faculty of Health Care Sciences, Himeji Dokkyo University, 7-2-1 Kamiono, Himeji 670-0896, Japan

5 Department of Otorhinolaryngology, Nagoya University Graduate school of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya 466-8550, Japan

6 Department of Health Science, Aichi Gakuin University, 12 Araike, Iwasaki-cho, Nisshin 470-0131, Japan

7 Research Division of Dementia and Neurodegenerative Disease, Nagoya University Graduate School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya 466-8550, Japan

Correspondence to: Drs. Sobue and Katsuno

Gen Sobue, MD

Research Division of Dementia and Neurodegenerative Disease, Nagoya University Graduate School of Medicine, Nagoya, Japan

65 Tsurumai-cho, Showa-ku, Nagoya 466-8550, Japan

TEL: +81-52-744-2943; FAX: +81-52-744- 2967; E-mail: sobueg@med.nagoya-u.ac.jp

Masahisa Katsuno, MD

Department of Neurology, Nagoya University Graduate School of Medicine

65 Tsurumai-cho, Showa-ku, Nagoya 466-8550, Japan

TEL: +81-52-744-2385; FAX: +81-52-744-2384; E-mail: ka2no@med.nagoya-u.ac.jp

Running title: Videofluoroscopic markers in SBMA

Key words: videofluoroscopic swallowing study; spinal and bulbar muscular atrophy; Kennedy's disease; biomarker; dysphagia

Abbreviations: ALSFRS-R = revised amyotrophic lateral sclerosis functional rating scale; SBMA = spinal and bulbar muscular atrophy; VFSS = videofluoroscopic swallowing study

47 references, 3 figures, 5 tables and 7 supplementary tables.

Word count: Title, 76 characters; Abstract, 398 words; Text, 4988 words

Abstract

Difficulty swallowing (dysphagia) is a major symptom of neuromuscular diseases, and often leads to life-threatening events such as aspiration pneumonia and suffocation. Although bulbar dysfunction is an important prognostic factor, reliable outcome measures have not been fully established to evaluate dysphagia in neuromuscular diseases. Spinal and bulbar muscular atrophy (Kennedy's disease) is a neuromuscular disease characterized by weakness of limb, facial, and oropharyngeal muscles. Patients have expanded CAG repeats in the androgen receptor gene. We aimed to clarify the characteristics of dysphagia in spinal and bulbar muscular atrophy using a videofluoroscopic swallowing study and to identify the plausibility of videofluoroscopic outcome measures for quantitative analysis.

A videofluoroscopic swallowing study was performed on 111 consecutive genetically confirmed patients with spinal and bulbar muscular atrophy and 53 age- and sex-matched healthy controls. Swallowing of 3 mL liquid barium was viewed in the lateral plane and was analysed by two independent evaluators using Logemann's videofluorographic examination of swallowing worksheet. We investigated which videofluoroscopic findings are critical to differentiate patients and controls, and between patients with and without subjective dysphagia.

Of more than 40 radiographic symptoms, the most pertinent abnormal findings in patients with spinal and bulbar muscular atrophy included *Vallecular residue after swallow* (residue just behind the tongue base)*, Nasal penetration,* and *Insufficient tongue movement* ($P < 0.001$ for each) compared with healthy controls. Quantitative analyses showed *Pharyngeal residue after initial swallowing, Oral residue after initial swallowing,*

Multiple swallowing sessions, and *Penetration–aspiration scale* were significantly worse in patients ($P \leq 0.005$ for each) compared with controls. In patients with spinal and bulbar muscular atrophy, laryngeal penetration was observed more frequently in patients without subjective dysphagia. Quantitative measures corresponded well with qualitative abnormalities but had limitations in reproducibility.

The qualitative and quantitative analyses of 111 patients showed that dysphagia of spinal and bulbar muscular atrophy is characterized by impaired tongue movement in the oral phase and nasal penetration followed by pharyngeal residues, which resulted in multiple swallowing sessions and laryngeal penetration. Given that the reproducibility and validity of videofluoroscopic data were partly compensated using the average values of three consecutive 3-mL barium swallows in our ad hoc reproducibility study, *Pharyngeal residue after initial swallowing* and *Penetration-aspiration scale* could be potential outcome measures in clinical trials. All patients, including those without subjective dysarthria, would be advised to practice the chin-tuck maneuver and the effortful swallow to reduce pharyngeal residues and penetration risk.

Introduction

The nervous system precisely controls the swallowing function in humans. Many neurological diseases give rise to difficulty swallowing (dysphagia) and aspiration pneumonia, which directly impact a given patient's prognosis. Since neurological diseases vary by affected nervous systems, each disease has a different mechanism and clinical phenotype of dysphagia. To date, dysphagia caused by some neurological diseases such as Parkinson's disease, Guillain–Barré syndrome, multiple sclerosis, and cerebrovascular diseases can be partially treated by medication and rehabilitation. Effective interventions, however, for dysphagia are scarce and are highly sought after for neuromuscular diseases, especially motor neuron diseases.

Spinal and bulbar muscular atrophy (SBMA), also known as Kennedy's disease, is a neuromuscular disease with extensive loss of lower motor neurons characterized by muscle atrophy, weakness, dysarthria, and dysphagia (Kennedy *et al*., 1968; Sperfeld *et al*., 2002; Sobue *et al*., 2011). SBMA exclusively affects males in their thirties or forties and the disease progression is slow. Early manifestations are typically hand tremor and muscle weakness. Approximately 5 years after initial signs of muscle weakness, affected patients require the use of a handrail for stairs and also dysarthria develops. Dysphagia develops in these patients after about 10 years of muscle weakness, followed by the need to use a cane and a wheelchair (Atsuta *et al*., 2006). The main cause of death in patients with SBMA is pneumonia due to bulbar palsy; dysphagia due to bulbar palsy is an important prognostic factor for SBMA.

The molecular basis of SBMA is the expansion of a trinucleotide CAG repeat, which encodes a polyglutamine tract, in the androgen receptor gene (La Spada *et al*., 1991).

Several studies have shown that CAG repeat size correlates with the age of onset in polyglutamine diseases, including SBMA (Atsuta *et al*., 2006; Hashizume *et al*., 2012). In SBMA, autopsy findings have shown that lower motor neurons were markedly depleted through all spinal segments, and neurons in the hypoglossal, facial, and trigeminal motor nuclei were severely depleted or atrophic (Sobue *et al*., 1989). Immunohistological staining shows abnormal protein deposits in the nuclei of remaining neurons, which were caused by lengthened CAG repeats (Adachi *et al*., 2005; Banno *et al*., 2012). Androgen deprivation therapy using an LH-RH agonist, leuroprorelin, decreases abnormal protein deposits and attenuates neurodegeneration in transgenic mice model of SBMA (Katsuno *et al*., 2003). The potential effect of this therapy has also been suggested in clinical trials (Banno *et al*., 2009; Katsuno *et al*., 2010).

Clinical markers reflecting disease processes are necessary for the design of clinical trials in neurological diseases as endpoints. Despite the importance of the swallowing function in patients' prognosis, reliable clinical markers to assess dysphagia have not been established. To investigate dysphagia in SBMA patients has several advantages. First, the disease mechanism is known to be a single cause, CAG repeat expansion, which would be ideal for accurate diagnosis and associated with less phenotypic heterogeneity than other neurological disorders such as amyotrophic lateral sclerosis (ALS). Second, most patients with SBMA are without apparent cognitive dysfunction leading to pseudobulbar palsy. Third, topographical simplicity of affected neurons merits understanding the characteristics and pathophysiology of dysphagia. Here, we investigated the findings of a videofluoroscopic swallowing study (VFSS) in patients

with SBMA to clarify the mechanism of dysphagia and to identify the plausibility of VFSS-based outcome measures for quantitative analysis.

Materials and methods

Participants

All the participating patients with SBMA and the healthy control participants underwent VFSS at Nagoya University Hospital from August 2003 to September 2010. VFSS reading was performed from November 2009 to February 2011. We also evaluated the backgrounds of the participants and their motor functions (CAG repeat length, ALSFRS-R etc.) at the time of the VFSS. None of the SBMA participants had had leuprorelin acetate for the treatment of dysphagia at the time of the VFSS. None of the control participants had any medical history of dysphagia or dysphagia-related diseases. All participants with SBMA gave their written informed consent for the genetic analyses.

Videofluoroscopic swallowing study (VFSS)

In the VFSS, participants were instructed to swallow 3 mL of 40% w/v barium sulphate twice in a standing position, as viewed in the lateral plane. To eliminate unnecessary irradiation exposure, the irradiation area was confined to the oral, pharyngeal, laryngeal, and upper esophageal areas for all participants. Each swallowing was recorded and included at least 20 seconds after the initial swallow. VFSS data were recorded on MiniDV digital videotape (Sony, Japan) at 30 frames per second. We analysed quantitative and qualitative findings on VFSS in consecutive patients with SBMA and healthy age- and sex-matched controls. All parameters were measured by two

independent evaluators (MD and SLP), who were blinded to details of the participants' backgrounds according to the standard procedures (Logemann *et al*., 1989; Logemann *et al*., 2000).

Qualitative and quantitative findings from the swallowing examination were measured using Logemann's videofluorographic examination of swallowing worksheet which contains more than 40 qualitative and quantitative radiographic symptoms. The two evaluators discussed and agreed on the details of the definitions before the measurement in accordance with the definition of each symptom, as described previously (Logemann, 1998).

Qualitative analyses

Qualitative items consisted of 6 items in the preparation to swallow; 15 items in the oral phase; 15 items in the pharyngeal phase; and 3 items in the cervical esophageal phase (Supplementary Table 1). The radiographic symptoms were read by the two evaluators independently and any discrepancies between the two evaluators were discussed later and found to be the same findings. If there was more than one positive radiographic symptom in two 3-ml-swallowings, then the swallowing test was considered positive for qualitative analyses.

Quantitative analyses

For quantitative analyses, the oral and pharyngeal residues were measured using semi-quantitative scales: 0, 2, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100%. Quantitative oral and pharyngeal barium residues were measured solely by the first 3 mL swallowed

because the first residue directly affects the second one. To assure intra- and inter-rater reliabilities, we trained the evaluators for this method. The concordance rates of quantitative measurements by two evaluators in the middle of, and again after, the study were 0.95, and 0.90, respectively. Given that piecemeal deglutition, a VFSS finding of multiple repeated swallows to empty a bolus from the oral cavity, is often observed in patients with SBMA, we measured pharyngeal residues *after initial swallowing* as well as those *after piecemeal deglutition*. We also measured times (sessions) of piecemeal deglutition for the first 3-mL barium swallow. Temporal measurements were evaluated as follows. The stage transition duration (STD), also known as pharyngeal delay time, was defined as the interval from the bolus passing the base of the tongue to the onset of laryngeal elevation, whereas the duration of maximum laryngeal elevation (laryngeal elevation duration; LED) was the length of time during which the larynx was maximally elevated from its rest position. The duration of cricopharyngeal opening (DCPO), also known as the duration of opening of the upper esophageal sphincter (DOOUES or DOUES), was defined as the length of time during which the cricopharyngeal sphincter was open. The duration of oral transit duration (OTD) was defined as the length of time from initiation of the posterior bolus movement in the oral cavity to arrival of the bolus at the ramus of the mandible. The pharyngeal transit duration (PTD) was defined as the length of time from the arrival of the head of the bolus at the ramus of the mandible to the tail of the bolus through the upper esophageal sphincter (Priefer and Robbins, 1997). The mean values of the first and the second doses of barium sulphate (3 ml) were calculated for all the temporal parameters of the VFSS. For the first swallow, the value of oropharyngeal swallowing efficiency (OPSE) was calculated as the percentage of the

bolus swallowed into the esophagus divided by the oropharyngeal transit time (OTD + PTD). OPSE is a summary measure of swallow function because it has been found to correlate with the degree of impairment in five patient populations including patients with oropharyngeal cancer, laryngeal cancer, and stroke (Rademaker *et al*., 1994).

Penetration-aspiration scale

For aspiration and penetration findings, we used a penetration-aspiration scale, an 8-point scale to describe penetration and aspiration events. Scores are determined primarily by the depth to which material passes into the airway and by whether or not material entering the airway is expelled (Score 8 being the worst aspiration). Acceptable intra- and inter-rater reliability for the scale has been established, with sufficient validity to support its introduction into clinical practice (Rosenbek *et al*., 1996).

Functional severity score

At the time appointed for the VFSS, we also administered the revised amyotrophic lateral sclerosis functional rating scale (ALSFRS-R). The ALSFRS is a validated questionnaire-based scale that measures physical function in patients with amyotrophic lateral sclerosis performing activity of daily living (The ALS CNTF treatment study (ACTS) phase I-II Study Group, 1996). The revised version of the ALSFRS was designed to improve the disproportion of weighting to the limbs and bulbar system as compared with respiratory dysfunction (Cedarbaum, 1999). The ALSFRS-R was translated into the Japanese language and validated (Ohashi *et al*., 2001).

The ALSFRS-R consists of 12 items: speech, salivation, swallowing, handwriting, cutting food and handling utensils, dressing and hygiene, turning in bed and adjusting bed clothes, walking, climbing stairs, dyspnoea, orthopnoea, and respiratory insufficiency. We used the total score and the sum of three bulbar-related subscores (speech, salivation, and swallowing) for analysis. The swallowing subscore was used to judge the presence of the patient's subjective dysphagia. We defined overt subjective dysphagia as a score of less than 4 (normal eating habits) on the swallowing subscore of the ALSFRS-R.

Genetic analysis

We extracted genomic DNA from peripheral blood of the SBMA patients using conventional techniques. We carried out PCR amplification of the CAG repeat in the androgen receptor gene using a fluorescent-labeled forward primer (5'-TCCAGAATCTGTTCCAGAGGTGC-3') and a non-labeled reverse primer (5'-TGGCCTCGCTCAGGATGTCTTTAAG-3'). The details of the PCR conditions have been described previously (Doyu *et al*., 1992). Aliquots of the PCR products were combined with loading dye and separated by electrophoresis using an autoread sequencer (SQ-5500; Hitachi Electronics Engineering). The size of the PCR standards was determined by direct sequencing, as described previously (Doyu *et al*., 1992).

Statistics

Statistical analyses were performed with IBM SPSS Statistics, version 19 (IBM Japan). Descriptive variables such as the mean, standard deviation, and range were used to summarize the quantitative measures. For qualitative analyses, we used chi-square to find

the differences between SBMA patients and controls, and between the subjective dysphagia group and the group without subjective dysphagia. For quantitative measures, we analysed the data by Spearman's rank correlation and Student's *t* test. We considered *P* values less than 0.05 as significant and correlation coefficients *R* greater than 0.3 as strong. For the analysis of relationships between subjective dysphagia and penetration, the McNemar test was performed (Table 5).

Ethics

This study was conducted according to the Declaration of Helsinki and its amendments. Written informed consent was obtained from each participant. Participants were free to withdraw from the study at any time for any reason. The Ethics Committee of Nagoya University Graduate School of Medicine approved the study. Confidentiality was ensured by assigning a study code to each participant. All studies conformed to the ethical guidelines for human genome/gene analysis research and the ethical guidelines for clinical research endorsed by the Japanese government.

Ad hoc study

To evaluate the reproducibility and validity of the VFSS measurement for barium residues, consecutive patients with SBMA were enrolled in an ad hoc study from May 2010 to August 2010. All participants in this ad hoc study (n = 20) were also evaluated in our previously described main study (n = 111). Participants underwent a VFSS twice during a month. The first and second VFSS were conducted on different dates at intervals of 7 to 30 days. In the VFSS of the ad hoc study, the participants were instructed to

swallow 3 mL of 40% w/v barium sulphate three times and 10 mL barium sulphate once. To avoid unfavourable carryover of residues to the next barium swallows, participants were instructed to conduct 10 mL water swallowing once and saliva swallowing twice, after each swallowing of barium. Pharyngeal barium residue and times of piecemeal deglutition (multiple repeated swallows) were measured blindly by two independent evaluators (MD and SLP), according to the standard procedures described previously.

To evaluate the validity of the measurement, the modified Norris scale (Limb Norris score and Norris bulbar score), SBMA functional rating scale (SBMAFRS), SWAL-QOL symptom subscores, and Swallowing Disturbance Questionnaire-Japanese (SDQ-J) were conducted. The modified Norris scale is a rating scale for ALS, which consists of two parts: the Limb Norris score and the Norris bulbar score. The former has 21 items to evaluate limb function and the latter has 13 items to assess bulbar function. Each item is rated in four ordinal categories, and thus the best scores possible are 63 and 39, respectively. The original version has been translated into Japanese and validated (Oda *et al*., 1996). SBMAFRS is a validated 14-item severity score for SBMA patients, which focuses on swallowing dysfunction instead of respiratory dysfunction (as in the ALSFRS-R) (Hashizume *et al*., 2015). The best possible score is 56 and the worst possible score is 0. The SWAL-QOL is a 44-item questionnaire about the impact of swallowing on quality of life. We used the data of 14 symptom items from the scale focusing on subjective dysphagia for the present study (McHorney *et al*., 2002). The best possible score is 70 and the worst possible score is 0. The SWAL-QOL has been translated into Japanese and validated (Wada *et al*., 2003). The SDQ is another questionnaire used to evaluate swallowing function (Manor *et al*., 2007). The best possible score is 0.5 and the worst

possible score is 44.5. The SDQ was translated into Japanese and validated as the SDQ-J (Yamamoto *et al*., 2012). The SDQ-J is divided into three domains: oral deficits, pharyngeal deficits, and history of pneumonia. We analysed the data by intraclass correlation (1, 1) for reproducibility and Spearman's rank correlation for validity.

Results

A total of 111 SBMA patients (53.2 ± 10.4 years of age; range 27-81) and 53 healthy controls (50.8 ± 9.0 years of age; range 40-71) were included in the study (Table 1). All participants were Japanese males. The SBMA group and the control group were similar in age ($P = 0.16$). The characteristics of this study population, such as age, CAG repeat length, disease duration and ALSFRS-R scores, were similar to those of previous studies (Atsuta *et al*., 2006; Katsuno *et al*., 2010; Fernández-Rhodes *et al*., 2011; Hashizume *et al*., 2012). Fifty-three out of 111 of the SBMA group reported having subjective dysphagia as determined by the ALSFRS-R swallowing subscore. Patients with subjective dysphagia were significantly older, with later disease-onset, lower total ALSFRS-R score, and lower ALSFRS-R bulbar subscores (Table 1). On the contrary, CAG repeat length and disease duration were not statistically different between the subjective dysphagia group and those without subjective dysphagia.

Qualitative analyses

SBMA vs. control

We compared qualitative radiographic symptoms (findings) in patients in the SBMA group with those in the control group. Significant qualitative characteristics of VFSS findings in the SBMA group were: *Vallecular residue after swallow* (residue just behind the tongue base)*, Residue (stasis) in both pyriform sinuses* (residue in both sides of the laryngeal orifice)*, Piecemeal deglutition* (multiple sessions of swallowing)*, Poor epiglottic inversion, Residue (stasis) on tongue, Nasal penetration, Reduced anterior-posterior tongue movement, Reduced laryngeal closure, Residue throughout the pharynx, Coating on pharyngeal walls after swallow, Repetitive lingual rolling actions, Head backward tilt in swallowing, Residue at top of airway,* and *Head forward tilt in swallowing* (all were $P < 0.05$; Fig. 1, bold radiographic symptoms). More than 80% of patients in the SBMA group showed *Vallecular residue after swallow* (residue just behind the tongue base). *Uncontrolled bolus/ premature swallow, Residue (stasis) in both pyriform sinuses* (residue in both sides of the laryngeal orifice)*,* and *Piecemeal deglutition* (multiple sessions of swallowing) were noted in about 50% of the SBMA group. Ten out of 14 significantly abnormal findings seen in patients with SBMA were the *pharyngeal* phase radiographic symptoms.

SBMA with subjective dysphagia vs. without subjective dysphagia

Among the characteristic qualitative radiographic symptoms (findings) described above, several findings significantly more frequently found in patients with subjective dysphagia compared with those without subjective dysphagia: *Residue (stasis) in both pyriform sinuses* (residue in both sides of the laryngeal orifice)*, Piecemeal deglutition* (multiple sessions of swallowing)*, Poor epiglottic inversion, Residue (stasis) on tongue, Nasal*

penetration, Reduced anterior-posterior tongue movement, Residue throughout the pharynx, and *Repetitive lingual rolling actions* (All were $P < 0.05$; Fig. 2, bold radiographic symptoms). Half of the findings that differentiated the patients with and without subjective dysphagia were *oral* phase radiographic symptoms.

Clinical background and radiographic symptoms

Of the qualitative radiographic symptoms, 14 that were characteristic of SBMA participants were associated with older age, later disease-onset, lower total ALSFRS-R scores, and lower ALSFRS-R bulbar subscores, although laryngeal penetration-related findings (*Reduced laryngeal closure, Residue at top of airway*) were not strongly correlated with ALSFRS-R bulbar subscores ($P = 0.6$, $P = 0.11$ respectively; Supplementary Table 2). CAG repeat length and disease duration were irrelevant to VFSS qualitative radiographic symptoms.

Quantitative analyses

SBMA vs. control

Compared with participants in the control group, patients with SBMA had a significantly greater amount of *Pharyngeal residue after initial swallowing* (mean ± SD: control 6.6 ± 10.8%, SBMA 14.0 ± 18.2%, $P = 0.001$), more *Oral residue after initial swallowing* (control 3.8 ± 2.9%, SBMA 5.2 ± 4.7%, $P = 0.005$), more frequent *Piecemeal deglutition* (multiple sessions of swallowing) (control 1.2 ± 0.4 times, SBMA 1.5 ± 0.8 times, $P < 0.001$) and higher score on the *Penetration-aspiration scale* (control 1.1 ± 0.3, SBMA 1.4 ± 0.8, $P = 0.003$) (Table 2). On the contrary, *Pharyngeal residue after piecemeal*

deglutition, *Oral residue after piecemeal deglutition*, *Oro-pharyngeal swallowing efficiency (OPSE)* and all the time-related measures did not reflect any significant difference between participants in the control group and those in the SBMA group (Table 2).

SBMA with subjective dysphagia vs. without subjective dysphagia

Among the four significant quantitative parameters that were characteristic of SBMA, patients with subjective dysphagia showed a significantly greater amount of *Oral residue after initial swallowing* (subjective dysphagia group 6.7 ± 5.6%, group without subjective dysphagia 3.9 ± 3.3%, $P = 0.002$) and more frequent *Piecemeal deglutition* (subjective dysphagia group 1.7 ± 0.8, group without subjective dysphagia 1.2 ± 0.6%, $P = 0.001$) (Table 3). Both *Oral residue after initial swallowing* and *Piecemeal deglutition* are classified as oral and voluntary measures. In contrast, *Pharyngeal residue after initial swallowing* and *Penetration–aspiration scale*, classified as pharyngeal and involuntary measures, did not show any significant difference between the group with subjective dysphagia and those without.

These two oral quantitative parameters (*Oral residue after initial swallowing* and *Piecemeal deglutition*) correlated fairly well with ALSFRS-R bulbar subscores ($R = -0.39$ and -0.35, respectively), whereas *Pharyngeal residue after initial swallowing* and *Penetration-aspiration scale*, which suggest pharyngeal phase dysfunction ($R = -0.29$ and -0.18), did not (Table 4).

Laryngeal penetration

Penetration-aspiration scale results revealed no aspiration episodes in our study. However, out of the 111 patients with SBMA, laryngeal penetration was found in 29 participants (26%). Patients with laryngeal penetration showed older age and older disease onset, but ALSFRS-R total score and ALSFRS-R bulbar subscores were not different between patients with and without laryngeal penetration (P = 0.81, 0.60, respectively) (Supplementary Table 3).

Although not statistically significant, the *Penetration-aspiration scale* results tended to be higher in patients with SBMA who had no subjective dysphagia (Table 3). Counterintuitively, laryngeal penetration was noted more often in patients without subjective dysphagia (19/58 cases, 33%) than in patients with subjective dysphagia (10/53 cases, 19%), and this difference was statistically significant ($P < 0.001$) (Table 5).

Ad hoc study for reproducibility and validity of pharyngeal residues

A total of 20 consecutive SBMA patients (58.8 ± 12.0 years of age) were included in the ad hoc study (Supplementary Tables 4, 5). Reproducibility of pharyngeal residues was more favorable in the mean of three swallows than in the first swallows (3mL and 10 mL) assessed by intraclass correlation (Supplementary Table 6). External validity was evaluated by comparing quantitative videofluoroscopic measurements with various swallowing severity scores (ALSFRS-R bulbar subscores, SBMAFRS bulbar subscores, SWAL-QOL, SDQ-J). Compared with single swallows, better correlation coefficients were noted between pharyngeal residue after initial swallowing (the mean of three swallows) and swallowing-related severity scores, especially SDQ-J (Supplementary Table 7, upper table). No remarkable differences were found between the 3-mL barium

swallow and the 10-mL barium swallow in terms of external validity of pharyngeal residues (Supplementary Table 7).

Discussion

This study examined characteristics of dysphagia in 111 patients with SBMA as compared with 53 healthy control participants with the use of the VFSS. SBMA patients showed various pharyngeal and oral deficits in VFSS, in comparison with healthy controls. Of note, ALSFRS-R bulbar scores correlated with oral dysfunctions, but not with bolus penetration into the airway, in the SBMA group. Laryngeal penetration was significantly more frequent in the patients without subjective dysphagia.

Mechanism of dysphagia in SBMA

This study identified characteristic radiographic symptoms in the SBMA group as compared with the control group. These radiographic symptoms include: *Vallecular residue after swallow* (residue just behind the tongue base)*, Residue (stasis) in both pyriform sinuses* (residue in both sides of the laryngeal orifice)*, Piecemeal deglutition* (multiple swallowing sessions)*, poor epiglottic inversion, Residue (stasis) on tongue*, and *Nasal penetration*. These findings were found in more than 40% of the SBMA patients and more frequently observed than in control participants. Other less frequent but statistically significant findings were related to tongue movement dysfunction, pharyngeal residue, and bolus penetration into the airway.

Taken together, we speculate that the mechanisms of dysphagia in SBMA patients function as follows (Fig. 3). Dysphagia in SBMA stems from both *Dysfunctional tongue movement* and *Incomplete velar elevation/tongue atrophy*. Dysfunctional tongue movement leads to oral and pharyngeal residues, both of which eventually result in multiple swallowing sessions (*Piecemeal deglutition)* and *Laryngeal penetration*. Incomplete velar elevation and tongue atrophy lead to *Nasal penetration*, inevitably causing low pharyngeal pressure, which leads to pharyngeal residue (*Residue in vallecula and pyriform sinuses*), *Poor epiglottic inversion*/*Reduced laryngeal closure,* and finally *Laryngeal penetration*. Laryngeal penetration is defined as entry of material into the laryngeal vestibule but not below the true vocal folds (Robbins *et al*., 1992). Penetrations were reported to be significantly more frequent after 50 years of age in normal aging. For people younger than 50, 7.4% of swallows exhibited penetration, while for people 50 years of age and over, 16.8% of swallows showed penetration in the healthy population (Daggett *et al*., 2006). Despite the fact that laryngeal penetration can be seen in the healthy controls, participants without laryngeal penetration were reported to have the lowest risk of developing pneumonia. Furthermore, patients with laryngeal penetration, tracheobronchial aspiration, or silent tracheobronchial aspiration were, in increasing order of magnitude, significantly more likely to develop pneumonia than patients with normal swallowing (Pikus *et al*., 2003).

A previous report using fiberoptic endoscopy also found that patients with SBMA have pharyngeal residues (Warnecke *et al*., 2009). Using VFSS findings, our study supports these findings and further found potential mechanisms of dysphagia and aspiration in SBMA patients.

Clinical outcome measure

One of the goals of this study was to find reliable objective quantitative markers that reflect severity of bulbar palsy in motor neuron disease patients. Clinical biomarkers are important to make go or no-go decisions in clinical trials. In this SBMA patient cohort, we speculated that there was less burden of genetic and phenotypic heterogeneity than in an ALS patient cohort, which is one of the main hindrances to therapeutic development in motor neuron diseases. SBMA is a slowly progressive disease (Kennedy *et al*., 1968; Hashizume *et al*., 2012). Thus, long-term clinical trials are necessary to assess whether certain drugs can alter the natural disease progression or not by targeting clinical endpoints such as occurrence of aspiration pneumonia or becoming wheelchair-bound. Suitable surrogate endpoints, which reflect the pathogenesis and severity of SBMA, are substantial to assess the therapeutic efficacy in drug trials.

We previously reported tongue pressure could serve as a marker of swallowing function at an early stage of the disease (Mano *et al*., 2014). VFSS is particularly useful in characterizing dysphagia mechanisms because it allows visualization of the swallowing movement while tongue pressure assesses a part of the swallowing function. In this study, compared with controls, SBMA patients had significantly more *Pharyngeal residue after initial swallowing, Oral residue after initial swallowing, Piecemeal deglutition* (multiple swallowing sessions), and worse *Penetration–aspiration scale* results. *Oral residue after initial swallowing* and *Piecemeal deglutition* correlated well to subjective dysphagia in SBMA patients, which was essentially irrelevant to laryngeal penetration. At this point in our current study results, *Pharyngeal residue after initial*

swallowing and *Penetration-aspiration scale* could be candidates for outcome measure, even though longitudinal changes with the disease course of these measures should be clarified in the future and a ceiling effect of the penetration-aspiration scale has been reported (Ludlow *et al.,* 2007).

Pharyngeal barium residue has also been shown to correlate with the incidence of aspiration and quantitative scintigraphy data, suggesting that this measurement is a reliable tool to evaluate swallowing function in patients with neuromuscular disorders (Eisenhuber *et al*., 2002; Logemann *et al*., 2005). Pharyngeal residue has also been reported to be the most important VFSS variable in reflecting pharyngeal pressure measurements in both diseases and healthy participants (Pauloski *et al*., 2009). To measure pharyngeal residue more precisely, the Normalized Residue Ratio Scale, using image analysis software, was recently proposed (Pearson *et al.*, 2013). Previous studies have shown high intra- and inter-rater reliabilities (Kuhlemeier *et al*., 1998), although not much is known about the reproducibility of this parameter. To improve the shortcomings, we also evaluated the reproducibility and validity of pharyngeal barium residues and piecemeal deglutition. The results showed better reproducibility and validity in the average values of three consecutive 3-mL barium swallows.

We also evaluated temporal parameters, which did not reveal significant importance in reflecting dysphagia severity in this cohort. Historically, cricopharyngeal myotomy in patients with ALS who have upper esophageal sphincter spasms has been reported (Mills*.,* 1973; Lebo *et al*., 1976; Loizou *et al*., 1980). But most ALS patients were reported to maintain normal upper esophageal sphincter relaxation (Higo *et al*., 2002). In our study, a longer duration of cricopharyngeal opening (DCPO), in other words a longer upper

esophageal sphincter opening duration, was related to smaller amount of pharyngeal and oral residues in healthy controls, whereas comparable relationships were not found in the SBMA group (data not shown). Variance of compensatory mechanisms in SBMA patients may have contributed to the lack of relationships between pharyngeal residues and DCPO in SBMA, and also the lack of significant difference in DCPO between the SBMA group and control.

Strengths and limitations

VFSS is a direct investigational method to delineate both spatial and temporal characteristics of swallowing in a patient. In the present study, SBMA patients with subjective dysphagia had both qualitative and quantitative oral-phase VFSS abnormalities. We also found that risk of laryngeal penetration is not relevant to subjective dysphagia or ALSFRS-R scores in the SBMA group. Possibly, this paradoxical finding can be explained by the speculation that patients with subjective dysphagia tend to swallow carefully (slowly or with multiple sessions of swallowing), leading to low incidence of laryngeal penetration. To assess the severity of dysphagia, VFSS is a superior method in terms of visibility of dysphagia mechanism, penetration, and aspiration among several modalities. VFSS is also useful to evaluate the effect of adaptive methods like the chin-tuck maneuver, the supraglottic swallow, and the effortful swallow. Given that the pharyngeal residue is the most common finding in SBMA patients and penetration risk exists even in those without subjective dysphagia, we would recommend the chin-tuck maneuver and the effortful swallow as a safe method of swallowing for all the SBMA patients with dysphagia (Wheeler-Hegland *et al.*, 2009; Leigh *et al.,* 2015). We recently

reported that a 6-week head lift exercise may improve swallowing dysfunction as measured by tongue pressure (Mano et al. 2015). Reinforcement of compensatory swallowing mechanisms may lead to effective rehabilitation in dysphagia patients.

Limitations of VFSS include poor reproducibility and radiation exposure. Longitudinal change in VFSS findings, especially in pharyngeal residue in SBMA patients, is another major limitation (Hashizume *et al*., 2012), partly caused by poor reproducibility of VFSS findings. Measuring multiple swallowing and using the average values of three swallowing may be a better method to improve poor reproducibility in VFSS, given our small sample-size ad hoc data. Comparisons between the control group and the diseased group are crucial to establish valid VFSS markers, but due to radiation problems we limited the number of healthy controls as compared with the number of patients with SBMA in our main study and in our ad hoc study. We could not analyse direct relationships between VFSS findings and pneumonia since only one of the participants had dysphagia severe enough to experience pneumonia, which was partly because our cohort focused on ambulatory patients. Since swallowing is composed of serial motions of various bulbar musculatures, multiple modalities including VFSS, tongue pressure, patient-reported outcomes, and other devices are necessary for dysphagia assessment in SBMA patients. Invention of noninvasive portable devices that can continuously measure pharyngeal residue would be ideal in the future.

Conclusion

Our data suggest that ALSFRS-R bulbar subscores are not relevant to bolus penetration into the airway. Our data indicate that VFSS of SBMA patients is qualitatively

characterized by oral and pharyngeal barium residue and laryngeal penetration, resulting from tongue and pharyngeal weakness, as well as compensative responses including repetitive swallows. Among quantitative indices, both *Pharyngeal residue after initial swallowing* and *Penetration-aspiration scale* reflect the major features of dysphagia in SBMA. These findings will be applied to future clinical trials of pharmacological and physical interventions.

Acknowledgements

We wish to thank Drs. Yukio Ohmae, Fumiko Ohshima, and Mineka Yoshikawa for technical advice, and all the patients, healthy volunteers and their families for the participation in this study.

Funding

This work was supported by a Center-of-Excellence (COE) grant, a Grant-in-Aid for Scientific Research on Innovated Areas 'Foundation of Synapse and Neurocircuit Pathology' and Grants-in-Aid from the Ministry of Education, Culture, Sports, Science and Technology of Japan (Nos. 23390230, 23790986, 26293206, 15K15337); grants from the Ministry of Health, Labor and Welfare of Japan ; Core Research for Evolutional Science and Technology (CREST) from the Japan Science and Technology Agency (JST); The Uehara Memorial Foundation Research Fellowship; and The Toyoaki Scholarship Foundation. There were no other funding sources, and the investigators had sole discretion over study design, collection, analysis and interpretation of data, writing of the report and decision to submit it for publication.

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Figure legends

Figure 1 Results of qualitative analyses (SBMA group vs. control group).

We compared qualitative radiographic symptoms in SBMA patients with those in the control group. Significant qualitative characteristics of VFSS findings in the SBMA group are shown in bold. More than 80% of SBMA patients had vallecular residue after swallow (residue just behind the tongue base). About 50% of SBMA patients had uncontrolled bolus/ premature swallow, residue (stasis) in both pyriform sinuses (residue in both sides of the laryngeal orifice), and piecemeal deglutition (multiple swallowing sessions). Ten out of 14 of the findings that differentiate SBMA patients from the control group were pharyngeal phase radiographic symptoms. Statistical significance was analysed by chi-square test.

Figure 2 Results of qualitative analyses of SBMA patients (with vs. without subjective dysphagia).

Among SBMA-related qualitative radiographic symptoms, we compared characteristic findings of patients with subjective dysphagia and those of patients without subjective dysphagia. Significant differences between the two group (SBMA patients with and without subjective dysphagia) are shown in bold. Half of the findings which differentiate with from without subjective dysphagia group were oral phase radiographic symptoms. Statistical significance was analysed by chi-square test.

Figure 3 Putative scheme of dysphagia in SBMA patients.

Dysphagia in SBMA stems from both Tongue movement dysfunction and Incomplete velar elevation/tongue atrophy. Tongue movement dysfunction leads to oral and pharyngeal residues both of which eventually result in multiple swallowing sessions (Piecemeal deglutition) and Laryngeal penetration. Incomplete velar elevation/tongue atrophy leads to Nasal penetration, inevitably causing low pharyngeal pressure which leads to pharyngeal residue (Residue in vallecula and pyriform sinuses), Poor epiglottic inversion/Reduced laryngeal closure and finally Laryngeal penetration.

Table 1 Demographics of study population at baseline

Characteristic	SBMA		*P*-value	Total SBMA (*n* = 111) mean ± SD (range)
	With subjective dysphagia (*n* = 53) mean ± SD (range)	Without subjective dysphagia (*n* = 58) mean ± SD (range)		
Age (years)	55.8 ± 9.8 (33-81)	50.8 ± 10.4 (27-74)	**0.01**	53.2 ± 10.4 (27-81)
CAG repeat length (number)	47.9 ± 3.3[a] (40-57)	48.5 ± 3.5[b] (42-57)	0.39	48.2 ± 3.4[c] (40-57)
Disease duration (years)	11.3 ± 8.5 (1-57)	10.7 ± 7.5 (1-33)	0.71	11.0 ± 8.0 (1-57)
Disease onset (years)	44.5 ± 11.0 (14-66)	40.0 ± 12.2 (8-68)	**0.05**	42.2 ± 11.8 (8-68)
ALSFRS-R	39.3 ± 4.1 (29-47)	43.2 ± 3.0 (36-48)	**<0.001**	41.4 ± 4.0 (29-48)
ALSFRS-R bulbar subscores	9.7 ± 1.4 (5-11)	11.5 ± 0.8 (9-12)	**<0.001**	10.6 ± 1.5 (5-12)

[a]*n* = 48; [b]*n* = 56; [c]*n* = 104.

Abbreviations: ALSFRS-R = revised amyotrophic lateral sclerosis functional rating scale.

Table 2 Quantitative analyses of videofluoroscopic swallowing study

Characteristic	Control (*n* = 53) mean ± SD (range)	SBMA Total (*n* = 111) mean ± SD (range)	*P*-value
Pharyngeal residue after initial swallowing (%),	6.6 ± 10.8 (0-60)	14.0 ± 18.2 (0-75)	**0.001**
Oral residue after initial swallowing (%)	3.8 ± 2.9 (0-15)	5.2 ± 4.7 (0-30)	**0.005**
Pharyngeal residue after piecemeal deglutition (%)	6.1 ± 10.6 (0-60)	8.0 ± 9.6 (0-55)	0.26
Oral residue after piecemeal deglutition (%)	3.5 ± 2.8 (0-15)	3.9 ± 2.9 (0-15)	0.46
Piecemeal deglutition (times) (Times of multiple swallowing sessions)	1.2 ± 0.4 (1-2)	1.5 ± 0.8 (1-4)	**<0.001**
Oro-pharyngeal swallowing efficiency (%)	54.2 ± 17.7 (13-99)	59.8 ± 26.6 (4-132)	0.12
Stage transition duration (sec)	0.34 ± 1.13 (-0.58-8.07)	0.37 ± 0.84 (-0.19-4.83)	0.81
Laryngeal elevation duration (sec)	0.23 ± 0.07 (0.12-0.43)	0.23 ± 0.10 (0.09-0.63)	0.74
Duration of cricopharyngeal opening (sec)	0.39 ± 0.06 (0.30-0.54)	0.39 ± 0.07 (0.25-0.67)	0.83
Oral transit duration (sec)	1.03 ± 0.52 (0.30-2.70)	0.82 ± 0.66 (0.20-4.90)	0.47
Pharyngeal transit duration (sec)	0.78 ± 0.19 (0.49-1.31)	0.76 ± 0.35 (0.48-3.13)	0.75
Penetration-aspiration scale	1.1 ± 0.3 (1-2)	1.4 ± 0.8 (1-6)	**0.003**

Table 3 Quantitative analyses between patients with and without subjective dysphagia

Characteristic	SBMA		*P*-value
	With subjective dysphagia (*n* = 53) mean ± SD (range)	Without subjective dysphagia (*n* = 58) mean ± SD (range)	
Pharyngeal residue after initial swallowing (%)	17.3 ± 19.0 (0-70)	11.0 ± 17.0 (0-75)	0.07
Oral residue after initial swallowing (%)	6.7 ± 5.6 (0-30)	3.9 ± 3.3 (0-15)	**0.002**
Piecemeal deglutition (times) (Times of multiple swallowing sessions)	1.7 ± 0.8 (1-4)	1.2 ± 0.6 (1-4)	**0.001**
Penetration-aspiration scale	1.3 ± 0.7 (1-5)	1.5 ± 0.9 (1-6)	0.22

Table 4 Correlation between demographics and quantitative videofluoroscopic parameters in SBMA patients (*n* = 111)

Radiographic symptom	Age (years)	CAG repeat length[a] (number)	Disease duration (years)	Disease onset (years)	ALSFRS-R	ALSFRS-R bulbar subscores
Pharyngeal residue after initial swallowing	0.26 *P* = 0.01	0.07 *P* = 0.49	0.04 *P* = 0.68	0.25 *P* = 0.009	-0.24 *P* = 0.011	-0.29 *P* = 0.002
Oral residue after initial swallowing	0.24 *P* = 0.01	-0.07 *P* = 0.47	0.04 *P* = 0.67	0.18 *P* = 0.055	**-0.36** ***P* < 0.001**	**-0.39** ***P* < 0.001**
Piecemeal deglutition (times) (Times of multiple swallowing sessions)	**0.35** ***P* < 0.001**	-0.14 *P* = 0.16	0.06 *P* = 0.52	**0.32** ***P* = 0.001**	-0.22 *P* = 0.019	**-0.35** ***P* < 0.001**
Penetration-aspiration scale	0.20 *P* = 0.03	-0.16 *P* = 0.10	-0.13 *P* = 0.19	0.23 *P* = 0.013	-0.04 *P* = 0.65	-0.18 *P* = 0.06

[a] *n* = 104

Abbreviations: ALSFRS-R = revised amyotrophic lateral sclerosis functional rating scale.

Data are shown by Spearman coefficient.

Table 5 Relationships between subjective dysphagia and penetration

		Subjective dysphagia		
		+	-	
Penetration	+	10	19	29
	-	43	39	82
		53	58	111

McNemar test was significant. ($P < 0.001$)

Supplementary table 1 Qualitative measurement items in videofluoroscopic findings

Phase	Characteristics
Preparation to swallow	Cannot hold food in mouth anteriorly Cannot form bolus Premature bolus loss Material falls into anterior sulcus Material falls into lateral sulcus Abnormal hold position
Oral phase	Delayed oral onset of swallow Searching tongue movements Tongue moves forward to start swallow Residue (stasis) in anterior sulcus Residue (stasis) in lateral sulcus Residue (stasis) on floor of mouth Residue in midtongue depression Residue (stasis) on tongue Disturbed lingual contraction Incomplete tongue-palate contact Residue on hard palate Reduced anterior-posterior tongue movement Repetitive lingual rolling actions Uncontrolled bolus/premature swallow Piecemeal deglutition
Pharyngeal phase	Nasal penetration Pseudoepiglottis (total laryngectomy) Bony outgrowth from cervical vertebrae Coating on pharyngeal walls after swallow Vallecular residue after swallow Coating in depression on pharyngeal wall Residue at top of airway Penetration into airway entrance Poor epiglottic inversion Reduced laryngeal closure Aspiration during swallow Residue (stasis) in both pyriform sinuses Residue throughout the pharynx Head backward tilt in swallowing Head forward tilt in swallowing
Cervical esophageal phase	Esophageal-to-pharyngeal backflow Tracheoesophageal fistula Zenker’s diverticulum

Supplementary table 2 Demographics and qualitative videofluoroscopic findings in SBMA patients (*n* = 111)

Radiographic symptom	Age (years) mean ± SD	*P*	CAG repeat length [a] mean ± SD	*P*	Disease duration (years) mean ± SD	*P*	Disease onset (years) mean ± SD	*P*	ALSFRS-R mean ± SD	*P*	ALSFRS-R bulbar subscores mean ± SD	*P*
Vallecular residue after swallow	54.4 ± 10.6	**<0.01**	48.3 ± 3.5	0.71	11.5 ± 8.4	0.12	42.9 ± 12.5	**0.03**	40.9 ± 4.1	**<0.01**	10.5 ± 1.5	**0.01**
	46.9 ± 6.0		47.9 ± 3.1		8.3 ± 4.8		38.4 ± 6.4		43.8 ± 3.0		11.3 ± 1.0	
Residue (stasis) in both pyriform sinuses	55.7 ± 10.3	**0.01**	48.2 ± 3.7	0.92	10.5 ± 6.7	0.57	45.1 ± 11.8	**0.01**	40.6 ± 4.4	**0.05**	10.3 ± 1.6	**0.01**
	50.8 ± 9.9		48.3 ± 3.2		11.4 ± 9.0		39.4 ± 11.2		42.1 ± 3.6		11.0 ± 1.2	
Piecemeal deglutition	56.3 ± 9.8	**<0.01**	47.5 ± 3.1	**0.04**	11.1 ± 5.5	0.87	45.2 ± 11.8	**<0.01**	40.4 ± 4.3	**0.01**	10.0 ± 1.6	**<0.01**
	50.3 ± 10.1		48.9 ± 3.6		10.8 ± 9.8		39.3 ± 11.2		42.3 ± 3.6		11.2 ± 1.0	
Poor epiglottic inversion	55.7 ± 9.7	**0.03**	48.4 ± 3.9	0.69	11.0 ± 6.6	0.99	44.7 ± 11.5	0.06	40.2 ± 4.0	**0.01**	10.0 ± 1.6	**<0.01**
	51.4 ± 10.5		48.1± 3.1		11.0 ± 8.8		40.4 ± 11.8		42.2 ± 3.9		11.1 ± 1.2	
Residue (stasis) on tongue	57.1 ± 9.3	**<0.01**	47.2 ± 2.8	**0.01**	10.8 ± 6.3	0.86	46.4 ± 11.6	**0.03**	39.1 ± 4.3	**<0.01**	10.0 ± 1.7	**<0.01**
	50.7 ± 10.3		48.9 ± 3.7		11.1 ± 8.9		39.5 ± 11.2		42.8 ± 3.2		11.1 ± 1.0	
Nasal penetration	55.7 ± 11.0	**0.04**	48.8 ± 3.7	0.18	11.3 ± 9.3	0.74	44.4 ± 12.4	0.12	40.2 ± 4.1	**0.01**	10.2 ± 1.6	**0.02**
	51.6 ± 9.7		47.9 ± 3.2		10.8 ± 7.0		40.8 ± 11.2		42.1 ± 3.9		10.9 ± 1.3	
Reduced anterior-posterior tongue movement	53.4 ± 11.3	**0.87**	48.4 ± 2.9	0.7	12.1 ± 9.6	0.34	41.3 ± 11.6	0.59	39.0 ± 4.5	**<0.01**	9.8 ± 1.7	**<0.01**
	53.1 ± 10.0		48.2 ± 3.7		10.5 ± 7.1		42.6 ± 12.0		42.4 ± 3.4		11.0 ± 1.1	
Reduced laryngeal closure	56.2 ± 10.8	**0.04**	47.3 ± 4.2	0.1	9.4 ± 6.4	0.18	46.7 ± 12.9	**<0.01**	41.6 ± 4.0	0.73	10.5 ± 1.5	0.6
	51.8 ± 9.9		48.7 ± 3.0		11.6 ± 8.5		40.2 ± 10.7		41.3 ± 4.1		10.7 ± 1.4	
Residue throughout the pharynx	57.9 ± 10.6	**<0.01**	47.7 ± 4.0	0.35	10.5 ± 6.7	0.73	47.3 ± 12.5	**<0.01**	39.4 ± 4.5	**<0.01**	9.7 ± 1.8	**<0.01**
	51.4 ± 9.8		48.4 ± 3.2		11.1 ± 8.4		40.3 ± 11.0		42.1 ± 3.6		11.0 ± 1.2	
Coating on pharyngeal walls after swallow	58.9 ± 11.6	**<0.01**	47.6 ± 3.6	0.3	9.5 ± 4.9	0.31	49.4 ± 11.0	**<0.01**	40.3 ± 4.5	0.14	10.1 ± 1.7	**0.09**
	51.6 ± 9.5		48.4 ± 3.4		11.4 ± 8.6		40.2 ± 11.3		41.7 ± 3.9		10.8 ± 1.4	
Repetitive lingual rolling actions	56.1 ± 11.3	**0.16**	47.8 ± 2.3	0.49	10.0 ± 5.5	0.54	46.1 ± 12.5	0.1	37.1 ± 4.4	**<0.01**	9.1 ± 1.7	**<0.01**
	52.5 ± 10.1		48.4 ± 3.7		11.2 ± 8.4		41.3 ± 11.5		42.4 ± 3.2		11.0 ± 1.1	
Head backward tilt in swallowing	52.9 ± 9.6	0.9	48.3 ± 3.5	0.89	10.7 ± 6.3	0.87	42.2 ± 10.0	0.99	40.3 ± 4.4	0.18	10.0 ± 1.4	**0.03**
	53.2 ± 10.6		48.2 ± 3.4		11.0 ± 8.3		42.2 ± 12.2		41.6 ± 3.9		10.8 ± 1.4	
Residue at top of airway	57.3 ± 11.5	**0.06**	48.4 ± 3.4	0.85	9.2 ± 5.8	0.28	48.2 ± 12.6	**0.01**	39.6 ± 3.9	**0.03**	10.2 ± 1.6	0.11
	52.3 ± 10.0		48.2 ± 3.5		11.3 ± 8.3		40.9 ± 11.3		41.7 ± 4.0		10.7 ± 1.4	
Head forward tilt in swallowing	55.9 ± 10.8	**0.28**	48.0 ± 4.2	0.8	9.4 ± 6.6	0.42	46.5 ± 12.2	0.13	39.7 ± 4.3	**0.08**	10.3 ± 1.6	0.38
	52.8 ± 10.3		48.3 ± 3.3		11.2 ± 8.2		41.5 ± 11.7		41.6 ± 4.0		10.7 ± 1.4	

[a] *n* = 104

Upper line denotes positive-symptom patients. Lower line denotes negative symptom patients.

Abbreviations: ALSFRS-R = revised amyotrophic lateral sclerosis functional rating scale.

Supplementary table 3 Background differences between SBMA patients with and without penetration

Characteristic	SBMA		*P*-value
	With penetration (*n* = 29) mean ± SD (range)	**Without penetration (*n* = 82) mean ± SD (range)**	
Age (years)	56.4 ± 11.2 (28-74)	52.0 ± 9.9 (27-81)	**0.05**
CAG repeat length (number)[a]	47.4 ± 4.1[b] (42-57)	48.5 ± 3.1[c] (40-57)	0.13
Disease duration (years)	9.5 ± 6.7 (1-29)	11.5 ± 8.3 (1-57)	0.25
Disease onset (years)	46.8 ± 12.9 (19-68)	40.5 ± 11.0 (8-66)	**0.01**
ALSFRS-R	41.2 ± 4.0 (32-47)	41.4 ± 4.1 (29-48)	0.81
ALSFRS-R bulbar subscores	10.5 ± 1.6 (7-12)	10.7 ± 1.4 (5-12)	0.60

[a]*n* = 104; [b]*n*= 28; [c]*n* = 76

Abbreviations: ALSFRS-R = revised amyotrophic lateral sclerosis functional rating scale.

Supplementary table 4 Clinical features of 20 patients with SBMA (ad hoc study)

Demographic	mean ± SD (range)
Age (years)	58.8 ± 12.0 (37-73)
Disease onset (years)	43.5 ± 13.4 (20-66)
Disease duration (years)	15.3 ± 8.6 (6-37)
Total ALSFRS-R score	40.1 ± 3.6 (33-46)
ALSFRS-R bulbar subscores	10.1 ± 1.3 (8-12)
Limb Norris score	50.0 ± 8.5 (25-60)
Norris bulbar score	31.9 ± 4.3 (23-38)
SBMAFRS score	37.7±7.7 (20-50)
SBMAFRS bulbar subscores	14.0 ± 2.8 (9-19)
SWAL-QOL symptom subscores	51.9±9.9 (38-69)
Total SDQ-J score	9.7±7.7 (0.5-24.5)
SDQ-J oral score	3.3±3.0 (0-8)
SDQ-J pharyngeal score	6.4±5.2 (0.5-18.5)

Abbreviations: ALSFRS-R = revised amyotrophic lateral sclerosis functional rating scale; SBMAFRS = spinal and bulbar muscular atrophy functional rating scale; SWAL-QOL = a questionnaire on swallowing problems in daily life; SDQ-J = swallowing disturbance questionnaire-Japanese.

Supplementary table 5 Videofluoroscopic findings in ad hoc study (*n* = 20)

	3 mL barium first swallow mean ± SD (range)	**3 mL barium second swallow mean ± SD (range)**	**3 mL barium third swallow mean ± SD (range)**	**3 mL barium mean of three swallows mean ± SD (range)**	**10 mL barium first swallow mean ± SD (range)**
Pharyngeal residue after initial swallowing (%)	11.4 ±11.0 (2-50)	22.3 ±24.2 (2-90)	17.6 ±21.7 (2-90)	17.1 ±17.2 (2-65)	18.5 ±21.5 (1-70)
Pharyngeal residue after piecemeal deglutition (%)	8.7 ±6.3 (2-20)	15.6 ±15.4 (2-50)	11.3 ±9.3 (2-25)	11.9 ±9.3 (2-30)	6.8 ±4.9 (0-15)
Piecemeal deglutition (times) (Times of multiple swallowing sessions)	1.5 ±0.9 (1-4)	1.6 ±0.8 (1-4)	1.5 ±0.7 (1-3)	1.5 ±0.7 (1-3)	2.0 ±1.0 (1-4)

Supplementary table 6 Reproducibility of ad hoc study (n = 20)

	3 mL barium first swallow	**3 mL barium mean of three swallows**	**10 mL barium first swallow**
Pharyngeal residue after initial swallowing (%)	0.195 (-0.251-0.577)	**0.729 (0.439-0.882)**	**0.882 (0.731-0.951)**
Pharyngeal residue after piecemeal deglutition (%)	0.380 (-0.054-0.696)	**0.783 (0.536-0.907)**	0.655 (0.306-0.850)
Piecemeal deglutition (times) (Times of multiple swallowing sessions)	0.479 (0.068-0.754)	**0.731 (0.444-0.884)**	0.361 (-0.076-0.685)

Upper number denotes intraclass correlation (1, 1); lower number denotes 95% confidence interval.

Supplementary table 7 Validity of ad hoc study (*n* = 20)

	3 mL barium first swallow Pharyngeal Residue After Initial Swallowing	3 mL barium mean of three swallows Pharyngeal Residue After Initial Swallowing	10 ml barium first swallow Pharyngeal Residue After Initial Swallowing
ALSFRS-R Total score	-0.044 (0.855)	-0.273 (0.244)	-0.372 (0.106)
ALSFRS-R bulbar subscores	-0.041 (0.865)	-0.232 (0.325)	-0.249 (0.290)
Limb Norris score	0.226 (0.337)	0.085 (0.721)	-0.014 (0.954)
Norris bulbar score	-0.149 (0.529)	-0.203 (0.390)	-0.165 (0.486)
SBMAFRS Total score	0.073 (0.761)	-0.097 (0.684)	-0.203 (0.391)
SBMAFRS bulbar subscores	-0.042 (0.860)	-0.232 (0.325)	-0.267 (0.256)
SWAL-QOL Symptom subscores	-0.235 (0.319)	**-0.457 (0.043)**	-0.308 (0.187)
SDQ-J Total score	0.386 (0.093)	**0.509 (0.022)**	0.315 (0.175)
SDQ-J Oral subscores	0.326 (0.160)	**0.482 (0.031)**	0.297 (0.203)
SDQ-J Pharyngeal subscores	0.432 (0.057)	**0.559 (0.010)**	0.388 (0.091)

Supplementary table 7 (cont.)

	3 mL barium first swallow Pharyngeal Residue After Piecemeal Deglutition	3 mL barium mean of three swallows Pharyngeal Residue After Piecemeal Deglutition	10 ml barium first swallow Pharyngeal Residue After Piecemeal Deglutition
ALSFRS-R Total score	-0.034 (0.886)	-0.155 (0.513)	-0.141 (0.553)
ALSFRS-R bulbar subscores	0.053 (0.824)	-0.073 (0.759)	0.126 (0.597)
Limb Norris score	0.136 (0.569)	0.146 (0.540)	-0.105 (0.660)
Norris bulbar score	0.006 (0.981)	-0.004 (0.986)	0.269 (0.251)
SBMAFRS Total score	0.068 (0.775)	0.002 (0.992)	-0.035 (0.883)
SBMAFRS bulbar subscores	-0.021 (0.929)	-0.125 (0.599)	0.098 (0.682)
SWAL-QOL Symptom subscores	-0.265 (0.259)	-0.439 (0.053)	-0.129 (0.589)
SDQ-J Total score	0.328 (0.158)	**0.492 (0.028)**	0.066 (0.781)
SDQ-J Oral subscores	0.311 (0.182)	**0.449 (0.047)**	0.136 (0.567)
SDQ-J Pharyngeal subscores	0.346 (0.135)	**0.510 (0.022)**	0.093 (0.696)

Supplementary table 7 (cont.)

	3 mL barium first swallow Piecemeal Deglutition	**3 mL barium mean of three swallows Piecemeal Deglutition**	**10 ml barium first swallow Piecemeal Deglutition**
ALSFRS-R Total score	-0.107 (0.655)	-0.341 (0.141)	-0.307 (0.189)
ALSFRS-R bulbar subscores	-0.170 (0.472)	-0.398 (0.083)	**-0.461 (0.041)**
Limb Norris score	0.061 (0.797)	-0.173 (0.466)	-0.065 (0.786)
Norris bulbar score	-0.416 (0.068)	**-0.494 (0.027)**	-0.269 (0.252)
SBMAFRS Total score	-0.103 (0.666)	-0.355 (0.125)	-0.289 (0.217)
SBMAFRS bulbar subscores	-0.081 (0.733)	-0.334 (0.150)	-0.369 (0.109)
SWAL-QOL Symptom subscores	-0.122 (0.609)	-0.180 (0.447)	-0.426 (0.061)
SDQ-J Total score	0.162 (0.495)	0.155 (0.514)	0.318 (0.172)
SDQ-J Oral subscores	0.156 (0.511)	0.227 (0.336)	0.421 (0.065)
SDQ-J Pharyngeal subscores	0.224 (0.342)	0.188 (0.428)	0.320 (0.170)

Upper number denotes Spearman's *R*; lower number with parenthesis denotes *P* value.
Abbreviations: ALSFRS-R = revised amyotrophic lateral sclerosis functional rating scale; SBMAFRS = spinal and bulbar muscular atrophy functional rating scale; SWAL-QOL = a questionnaire on swallowing problems in daily life; SDQ-J = swallowing disturbance questionnaire-Japanese.

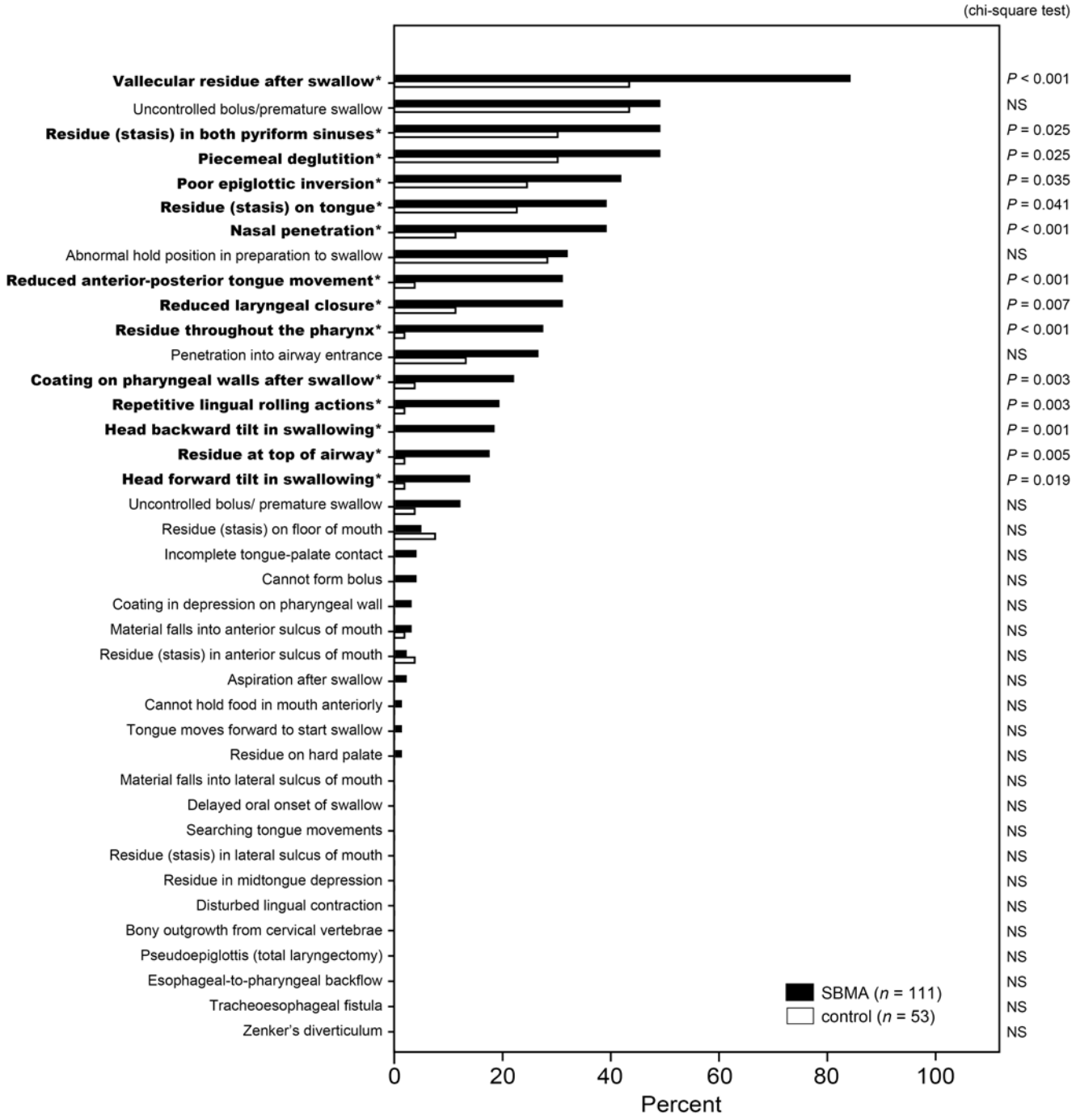

Figure 1 Results of qualitative analyses
(SBMA group vs. control group)

* Statistical significance was found by chi-square test

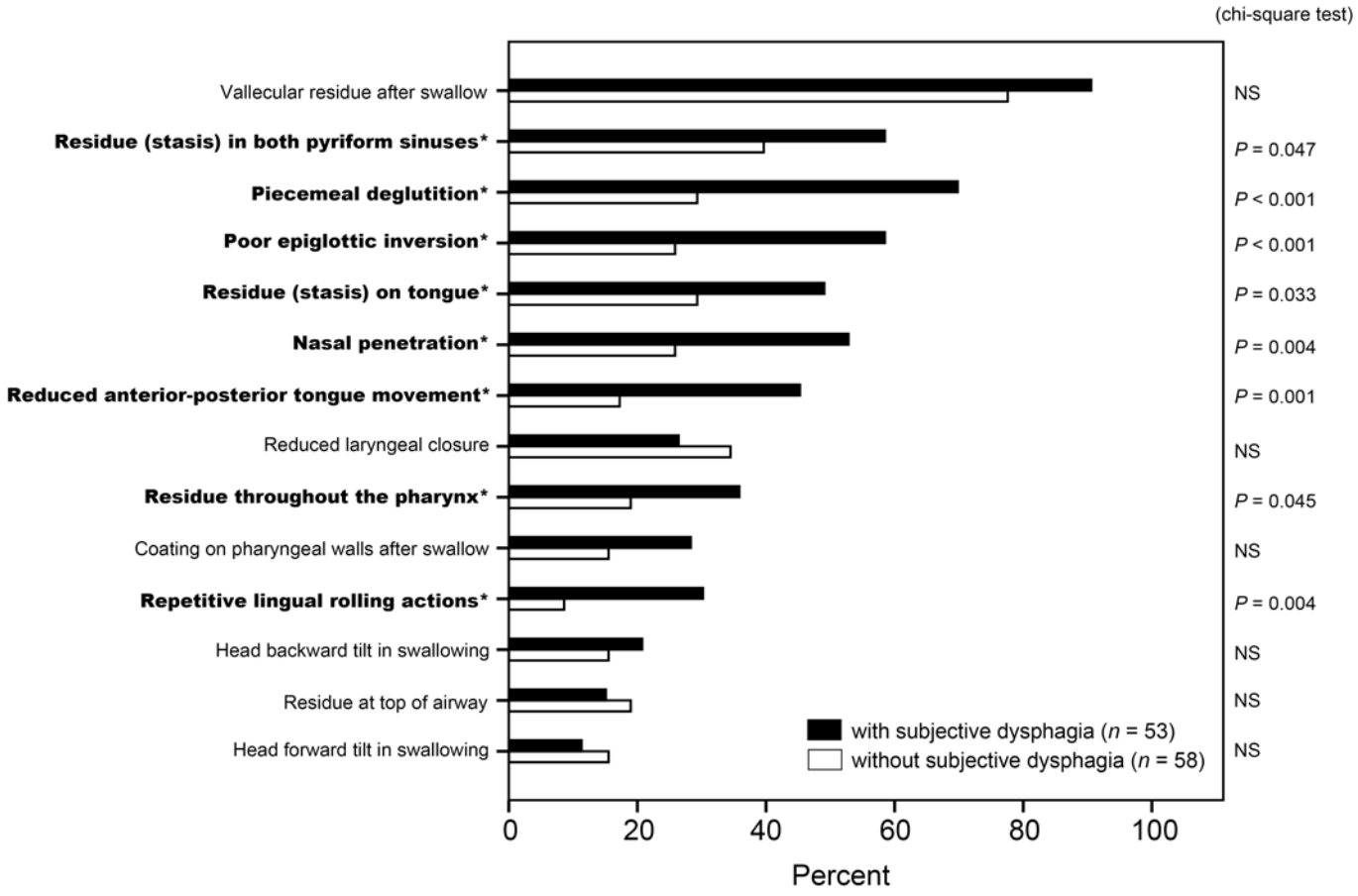

Figure 2 Results of qualitative analyses of SBMA patients (with subjective dysphagia vs. without subjective dysphagia)

* Statistical significance was found by chi-square test

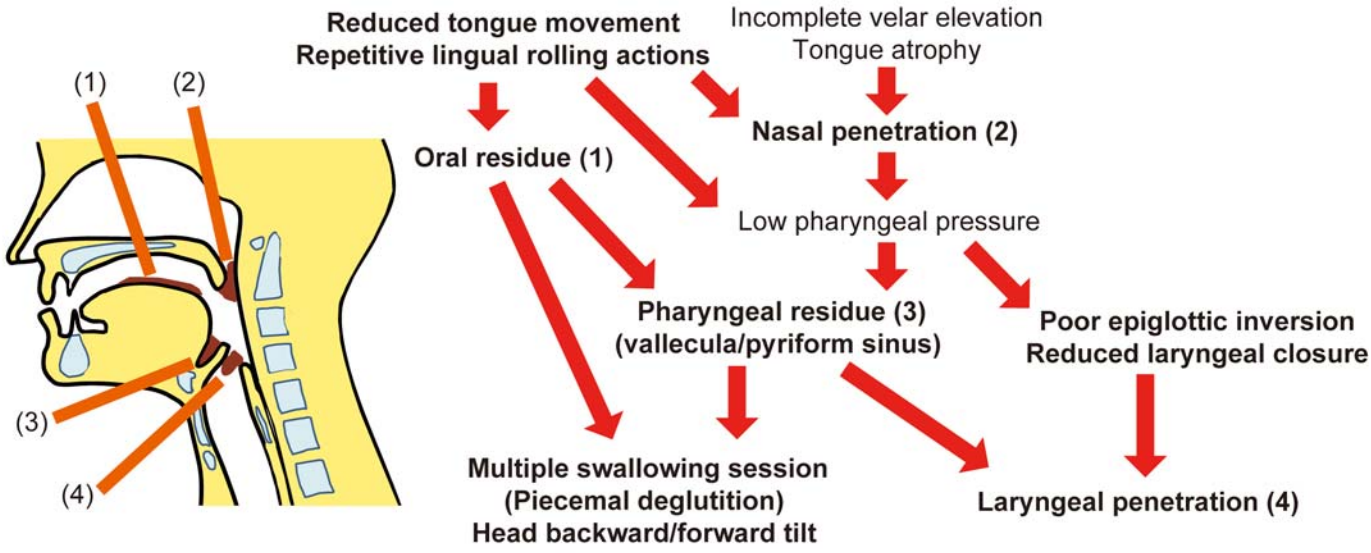

Figure 3 Putative scheme of dysphagia in SBMA patients

Acknowledgements

Merit Cudkowicz and Neurological Clinical Research Institute members:

James Berry, Nazem Atassi, Sabrina Paganoni, Elena Ratti, Katharine Nicholson

Gen Sobue and Lab members:

Masahisa Katsuno, Keisuke Suzuki, Atsushi Hashizume, Seiya Tanaka

MPCTI Program:

Jonathan Williams, Anthony Hollenberg, Paul Conlin, Lauren Dewey Platt

Ayako Toyama & Yukari Banno